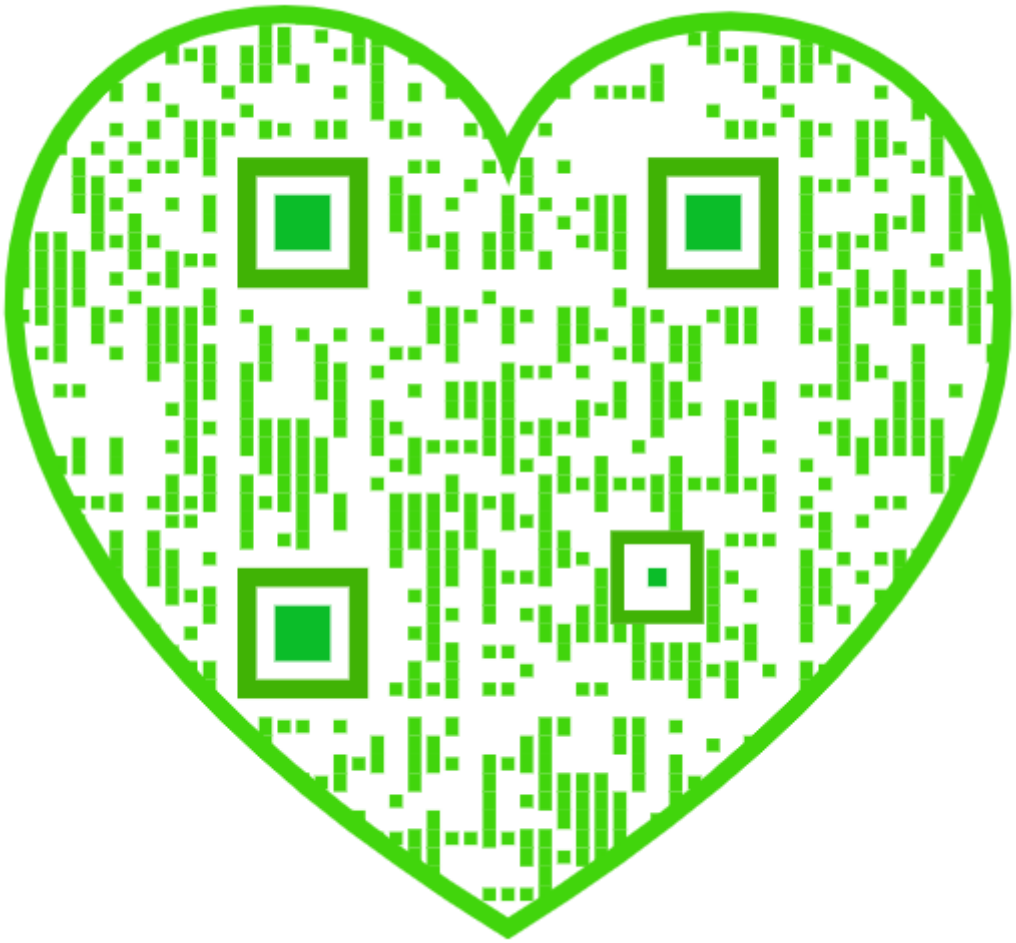

ANNALS OF BOTANY

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VOLUME XXII

With Thirty-five Plates, Fifteen Diagrams, and Ninety Figures in the Text

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Spore Formation in Derbesia.

BY

BRADLEY MOORE DAVIS.

With Plates I and II.

THE genus *Derbesia* described by Solier ('47) is chiefly remarkable for its zoospores. These are very large, and each is provided with a circle of numerous long cilia at its forward end. The general morphology of *Derbesia* is that of the Siphonales, with many points of resemblance to *Bryopsis*, except that the filaments are sparingly and irregularly branched. The zoospores of the Siphonales, however, are biciliate, *Vaucheria* excepted, and small. The peculiarities of the zoospores of *Derbesia* have led Blackman and Tansley ('02) to suggest that its affinities are widely different from *Bryopsis*, a point which will be considered later in the paper.

The zoospores of *Derbesia* offer, then, a very interesting subject for cytological study, since their large size and the peculiar arrangement of the cilia give promise of interesting details in the form and development of the blepharoplast. This interest is further enhanced by Berthold's account of their development. The zoospores are almost invariably uninucleate at maturity, but they are developed in relatively small numbers in sporangia which contain many thousands of nuclei when they are first formed from the parent filaments. Berthold ('81) reported that the larger nuclei, finally present one in each zoospore, are formed by the successive fusions of the very numerous nuclei within the sporangium. This is, I think, the last account of nuclear fusions of this character which has not been disproved by detailed cytological investigation, for similar accounts of nuclear fusions during oogenesis in the Saprolegniales, Peronosporales, and *Vaucheria* have been shown by later research to be incorrect, and such reductions in the number of nuclei within multinucleate reproductive cells have proved to be due in every case to nuclear degeneration rather than to nuclear fusions.

[Annals of Botany, Vol. XXII. No. LXXXV. January, 1908.]

This paper will describe the development and germination of the zoospores of *Derbesia Lamourouxii* (Agardh), Solier, with special reference to the structure and behaviour of the nuclei, and the development and fate of the blepharoplast. The material was gathered in the spring of 1904 at Naples, where, at the Zoological Station, I occupied a table of the Carnegie Institution.

The most satisfactory fixation was obtained with a weak chrom-osmo-acetic formula as follows:—1 per cent. chromic acid 25 c.c., 1 per cent. acetic acid 10 c.c., 1 per cent. osmic acid 5 c.c., sea water 60 c.c. A similar formula, with the omission of osmic acid, was also satisfactory. Sections were generally cut 3 μ thick. The best stain proved to be iron-alum haematoxylin with congo red, and haematoxylin alone for the details of nuclear structure. It proved somewhat difficult to use safranin and gentian violet because of the numerous plastids which take these stains with avidity.

The Habits of the Zoospores.

The zoospores are formed slowly, and several days may elapse after the development of the sporangium before the segmentation of the protoplasm begins. The first large crop generally appeared two or three days after material of *Derbesia* was brought into the laboratory and left in sea water, after which zoospores continued to be formed for several days. Attempts to hasten their development by raising the temperature of the water or by diluting it resulted in the death of the plants.

The zoospores in the Naples material were remarkably variable in size and in number, ranging from 30–50 in the smaller sporangia to perhaps 200–300 in the larger. They are spherical or oval, and swim slowly through the water with the circle of cilia forward (Pl. I, Fig. 1). They contain very large numbers of small disk-shaped chloroplasts distributed throughout the protoplast, so that the zoospores are uniformly green and the ciliated region is not conspicuously lighter in colour; there are no pigment spots.

The zoospores come to rest on the region of the protoplast within the circle of cilia, and the latter become distributed in a radiating arrangement (Fig. 2) over the surface to which the zoospores become attached.

The Development of the Sporangium.

The sporangia are globular structures arising from the sides of the filaments. It must require a number of days for them to reach maturity. The contents of the developing sporangium remain connected with the parent filament by a broad strand of protoplasm until the structure has attained full size (Fig. 3). The strand then becomes narrower by the

gradual formation of a thick ring at the base of the sporangium. Sections of this ring (Fig. 4) show that its substance (evidently cellulose in character) is laid down in concentric layers on the inside of the wall of the filament at the base of the sporangium. The protoplasm of the sporangium is thus gradually pinched off from that of the filament and is not separated by thin cleavage furrows as in the sporangia of the moulds.

The ring-like deposit thickens and finally becomes a heavy plate of cellulose (Fig. 5), which presents a laminate structure, showing that its substance is deposited in successive layers as the protoplasm in the sporangium and in the filament withdraw from one another. The wall shown in Fig. 5 is from a sporangium in which the protoplasmic cleavage to form the spores had already begun.

The Differentiation of the Nuclei in the Sporangium and the Degeneration of the Smaller Nuclei.

The nuclei which enter the developing sporangium are similar and all of about the same size and a little larger than the plastids (Fig. 6). Each contains a small deeply-staining nucleolus which frequently lies within a clearer circle. The remainder of the nuclear cavity is almost wholly occupied by a large body which stains lightly but is readily distinguishable. This body is probably chromatic in character as shown by its later history, but at this stage it is homogeneous and gives no indication of granular or fibrillar structure. There must be several thousand of these nuclei which enter the developing sporangium.

The nuclei begin to show a differentiation in size very shortly after the protoplasm of the sporangium becomes separated from that of its parent filament or even before this time. Certain of them increase in size and become conspicuous in the sporangium, finally reaching a diameter 4–6 times the size of the plastids (Fig. 8). The large nuclei may have smaller nuclei very near them, singly or in groups, to which they appear in sharp contrast (Fig. 7). The internal structure of the nucleus does not apparently change during its growth. The large homogeneous chromatin body, however, increases in size.

There are, however, marked changes in the appearance of the protoplasm around the enlarging nuclei. A granular cytoplasm forms an envelope so that the plastids come to lie at some distance from the nuclear membrane (Fig. 9), and delicate protoplasmic strands radiate out from the membrane between the plastids into the surrounding protoplasm (Fig. 9). These strands end in deeply-staining granules just outside of the nuclear membrane. The large nuclei are thus very prominent in the sporangia because of their size and the radiating strands from the enveloping zone of granular protoplasm bordered by the plastids.

The larger nuclei are distributed rather uniformly throughout the protoplasm within the sporangium, and they lie generally at some distance from one another. The protoplasm between is vacuolate and filled with plastids among which the smaller nuclei lie singly or in groups (Fig. 7). These nuclei at this time are of about the same size as when the protoplasm entered the developing sporangium, that is, about the size of the plastids, but some of them are a little larger and some smaller. They show a marked tendency to become massed in groups (Fig. 10) in regions of granular cytoplasm free from plastids.

There is no evidence that the smaller nuclei fuse with one another to form the larger. This point was studied with great care. These nuclei decrease in size, and shortly become much smaller than the plastids (Fig. 10). Although they may lie so close together as to be in actual contact, they never unite. The small nuclei are not easily found when they have become half the size of the plastids, but careful search will reveal them in larger and smaller groups and also singly, and the fully mature sporangium, shortly before spore formation, has probably just as many nuclei as when first formed. The small nuclei finally break down and are generally completely lost at the time when the spores are formed, except for deeply-staining globules, which are probably the remains of the nucleoli.

It is difficult to understand the reasons for this rapid differentiation of the nuclei in size. I was not able to discover any cytoplasmic structures, such as coenocentra, which could be interpreted as centres of dynamic or metabolic activity affecting the nourishment of nuclei in their vicinity, as appears to be the case during oogenesis in the Perisporiales and Saprolegniales. However, the arrangement of the protoplasm around the larger nuclei is very suggestive of important dynamic or metabolic relations between them and the cytoplasm in the immediate vicinity. The envelope of granular cytoplasm, which has the appearance of kinoplasm, and more especially the radiating protoplasmic strands, give to the nuclei the appearance of being themselves important centres of dynamic and metabolic activity. Their distribution also at relatively wide intervals throughout the protoplasm indicates that each is really the centre of a definite region of protoplasm in the sporangium. It is probable that the radiating strands are the paths of protoplasmic streams which pass to and fro between the large nuclei and the surrounding protoplasm. They are, therefore, probably the paths of dynamic and metabolic activities between the large nuclei and the regions of the protoplasm which they dominate.

It would seem as though there were an actual struggle for existence among the nuclei in the sporangium to control a limited amount of protoplasm with limited metabolic and dynamic possibilities, and that relatively few of the nuclei were successful while the majority perished. I have already suggested this explanation to account for the degeneration of the

nuclei in the oogonia of the Saprolegniales, Peronosporales, and *Vaucheria* (Davis, '03, p. 341, and '04). The physiological conditions within the oogonia of these groups and the sporangium of *Derbesia* are very similar. These reproductive organs are overstocked with nuclei in the beginning of their development, for reasons that are probably phylogenetic, and related to times when the organs developed many more reproductive cells than they do at present. After the reproductive organs are separated from the parent filaments the nuclei find themselves under conditions where there is not sufficient nourishment for all, and a struggle for existence develops with the subsequent degeneration of all the nuclei except the favoured few.

In the Saprolegniales and Peronosporales the survival of certain nuclei is determined by their favourable position near those metabolic centres in the cytoplasm, the coenocentra. In *Vaucheria* (Davis, '04) the surviving nucleus lies near the centre of the oogonium in an accumulation of cytoplasm which is probably the region of the cell most favourable for nuclear metabolism. The sporangium of *Derbesia* is so large a ccenocyte that its cytoplasm would not be expected to become uninucleate. However, regions become differentiated and dominated by certain nuclei which find there more favourable conditions for growth, and consequently obtain a lead over all others, finally forcing their degeneration. The positions of these favoured centres of metabolic activity are marked by the accumulation of granular cytoplasm around the large nuclei and the protoplasmic strands radiating out among the plastids.

The last stages in the degeneration of the small nuclei are more easily followed in *Derbesia* than in *Saprolegnia, Albugo*, or *Vaucheria*, because the nuclei are not so minute as in the latter forms. The nuclei decrease in size as shown in Fig. 10 until they are less than half the diameter of the plastids. The nucleolus still stains distinctly, but the chromatin body becomes faint and is finally quite lost, so that the nucleolus alone comes to lie in a vacuole which is the remains of the nuclear cavity. The boundary of the vacuole finally breaks down, and the nucleolus then passes into the cytoplasm as a deeply-staining globule. Fig. 11 shows a group of degenerating nuclei in a region of granular cytoplasm surrounded by plastids. This group is in an advanced state of degeneration. Some of the nuclei still have the nuclear membrane, but the globules lying freely in the cytoplasm are from those that have become disorganized. These nucleoli fragment into smaller globules, which finally disappear.

The Segmentation of the Protoplasm.

The segmentation of the protoplasm does not begin until the process of nuclear degeneration is practically ended. Traces of the smaller nuclei may still be found, but they are very difficult to recognize. The sporangium then contains only the larger nuclei quite uniformly distributed through the

protoplasm. Segmentation takes place by cleavage furrows which start at the periphery of the protoplast within the sporangium and cut into the protoplasm in the form of curved and branching furrows (Fig. 12).

The cleavage furrows first mark out fairly large areas which may contain a number of nuclei, but the areas become successively smaller as new furrows are formed at the periphery or strike off from the sides of the older into the protoplasm. Finally, the furrows so divide the protoplasm that it becomes blocked out into approximately equal, uninucleate masses (Fig. 13), and these gradually round up as the zoospore originates. The zoospores are almost invariably uninucleate, but I have found binucleate examples (Pl. II, Fig. 27) with blepharoplasts fully developed. These undoubtedly result from conditions similar to the formation of bi- and tri-nucleate eggs of *Saprolegnia* and some species of *Albugo* (Davis, '03, pp. 243 and 324), i. e. two or more nuclei have been able to exist sufficiently near one another to be included in the same area of protoplasm when the reproductive cells were formed.

The plastids in the zoospore-origins and young zoospores (Fig. 14) are arranged about the nuclei with their longer axis radiating outward, so that the nucleus has very conspicuously the appearance of being the centre of the cell not only geometrically but also dynamically, as shown by the finer structure of the protoplasm. The radiating strands from the cytoplasm investing the nucleus are prominent, each with a granule as its base. The protoplasmic strands run out into the cytoplasm between the plastids, but the latter are so densely crowded that it is impossible to trace the strands far, and I was not able to determine whether they reach the periphery of the zoospore and thus form a radiating mesh between the nucleus and the outer plasma membrane. It seems probable, however, that they do so.

The Development of the Blepharoplast and its Cilia.

The blepharoplast of the zoospore of *Derbesia* is at maturity a remarkable structure. It consists of a double ring (Fig. 22) situated just underneath the plasma membrane. The cilia are developed from the lowermost of the two rings and radiate out so as to form a circle or crown at one end of the zoospore (Fig. 1). The blepharoplast is generally about one and a half times or twice the diameter of the nucleus. The study of such a large and remarkably shaped structure presented exceptional opportunities for the investigation of some disputed points concerning the origin and relation of this cilia-forming organ to the nucleus and the outer plasma membrane of the cell.

Previous to the formation of the blepharoplast the nucleus moves from near the centre of the zoospore-origin towards the periphery. It is then clear that the radiating strands on the side of the nucleus nearest the

periphery are actually connected with the outer plasma membrane (Fig. 15). About one-third or one-fourth of these strands may be so connected, while the remainder, as before, radiate out into the cytoplasm between the plastids.

At this time granules may be found on the strands at various distances between the nucleus and the plasma membrane (Fig. 15). The sharpness of the granules indicates that they are organized bodies travelling along the strands, which become arranged in a funnel-shaped form with the broadest region of the funnel at the periphery of the cell. It is, however, possible that what appear to be granules are in the living cells merely thickenings of the strands, and that material moves outwards from the periphery of the nucleus through the strands in a semifluid condition and not as granules; but the sharpness of form and depth of staining of these structures indicate that definite granules are actually present. The appearance of the funnel-shaped arrangement of the strands in relation to the nucleus when viewed from the interior of the zoospore is shown in Fig. 16. The granules accumulate in a circle just underneath the plasma membrane where they later form the blepharoplast. The funnel-shaped group of strands connecting the nucleus with the periphery seems to exert a pull on the plasma membrane so that it is drawn inward as a slight depression or shallow groove.

The granules gradually appear more numerously in the circle just underneath the plasma membrane and with the nucleus lying below (Fig. 17). A portion of such a circle, viewed from the interior of the zoospore, is shown in Plate II, Fig. 18. The granules then fuse with one another to form a ring, which becomes the blepharoplast, staining very black with haematoxylin, and so firm in texture that it may be sharply cut with the microtome knife. Its appearance when viewed from the surface of the zoospore is shown in Fig. 19. The strands connecting the nucleus with the developing blepharoplast become less conspicuous and finally disappear after the blepharoplast is formed, leaving the boundary of the nucleus nearest to the plasma membrane without conspicuous radiations. The nucleus then passes from the periphery back to the centre of the zoospore, and the plastids become distributed around it as before in a radiating arrangement (Fig. 20). The plastids also encroach into the region of the zoospore just under the blepharoplast from which they had previously been excluded by the funnel-shaped group of protoplasmic strands.

The blepharoplast when first formed is a single ring as shown in cross-section (Fig. 21 *b*). It lies very close to the outer plasma membrane, but is not a part of it. The blepharoplast now splits into two rings, one below the other, and the circle of cilia grows out from the lower (Fig. 22). The splitting of the primary ring is a gradual process. The ring changes its form so that in cross-section it appears somewhat U-shaped with the arms of the U very much thickened (Fig. 23 *b*). The two thickened portions then separate

as two rings, one below the other, the lower slightly larger than the upper, so that it appears somewhat outside when the blepharoplast is viewed from above (Figs. 22, 24).

The cilia, as stated before, arise as outgrowths from the lower ring (Figs. 22, 25, 26). It was not possible to determine whether each cilium was connected with a definite granule such as entered into the composition of the blepharoplast, for the latter has the appearance of being an homogeneous structure. It stains evenly, and the granules that formed it (Fig. 18) fuse together so completely that their individuality becomes lost. The splitting of the primary ring is a very curious process, for which I have no explanation to offer.

Mention has been made of the occasional binucleate spore origins. A section of such a one is shown in Fig. 27, so far advanced in its development that its blepharoplast is formed. There can hardly be doubt but that such an example would give rise to a binucleate zoospore. The development of such a zoospore simply means that the progressive segmentation of the protoplasm by the branching cleavage furrows does not always proceed far enough to separate all of the large nuclei from one another.

During the development of the zoospores some important changes take place in the structure of the nucleus. As shown in Fig. 6, the nuclei which enter the sporangium contain two structures: (1) the deeply-staining relatively small nucleolus, and (2) a large homogeneous body which is chromatic in character, as proved by its later history, and which I have called the chromatin body. This latter structure remains unchanged in appearance during the differentiation of the nuclei in size (Fig. 7), but later, changes become apparent within the large nuclei. Delicate strands appear in the nuclear space, forming a loose network (Figs. 8, 10), and the chromatin body ceases to be homogeneous in structure but becomes instead granular or cloudy. Finally, the nuclei at the time of the segmentation of the protoplasm to form the zoospores (Figs. 12, 13) contain loose masses of cloudy material connected by strands to form an irregular network. While the blepharoplast is being developed the network becomes more and more conspicuous and stains more deeply (Figs. 16, 19). The cloudy masses at last practically disappear, and there are present in the nucleus coiled threads, generally somewhat gathered in the centre near the nucleolus (Figs. 21, 24). These coiled threads are clearly chromatic, and from them is organized the spirem of the first mitosis in the germinating spore.

Perhaps the most important result of the present study of *Derbesia* is the clear evidence that the nucleus is concerned in a most intimate manner with the development of the blepharoplast. The nucleus leaves its position in the centre of the cell, the radiating protoplasmic strands over a certain portion of its surface take a definite position in the form of

a funnel, and granules apparently pass outward to the plasma membrane. After the blepharoplast is formed the nucleus returns to the centre of the cell. This important work being accomplished, it is probable that the nucleus maintains connexions with the blepharoplast by very delicate protoplasmic strands, although such conditions would not be easily observed in *Derbesia* because of the numerous and crowded plastids. The characteristic clear areas at the tips of most zoospores of various algae indicate that these regions are occupied by protoplasm, which is kinoplasmic in character, and consequently likely to hold vital relations to the nucleus. It is clear that the blepharoplast of *Derbesia* is not a development from the plasma membrane, but from granules closely associated with protoplasm investing the nucleus.

The Germination of the Zoospores.

As has been stated, the zoospore comes to rest on the region of the protoplast within the circle of cilia, which spread out in a radiating arrangement over the surface to which the zoospore becomes attached (Fig. 2). The spore generally forms a small papilla at this region, which develops into a simple holdfast. The blepharoplast may be easily found during the earlier stages of germination. Fig. 28 (Plate II) shows the base of a germinating spore, the nucleus of which had not yet divided; the blepharoplast appears in section, and it should be noted that the two rings are much more delicate than in the zoospore, although the magnification is twice as great. The nucleus or nuclei in the germinating spores come to lie near the periphery of the cell because of the development of a central vacuole which forces the protoplasm to take position just under the cell wall. Fig. 29 shows the base of an older sporeling which contained several nuclei; the two rings of the blepharoplast are somewhat separated here. In both figures the blepharoplast is evidently pressed very close against the cell wall, which has been formed, and appears now to lie in the plasma membrane. The blepharoplast becomes fainter in older stages of germination, and can be found only with difficulty. It fades away, and probably at last entirely disappears.

The mitoses in the germinating spore are difficult of study because the nuclei are so small. It is, however, very clear that the resting nucleus contains besides the nucleolus a chromatin network (Fig. 30) in place of the chromatin body, which is so characteristic of the nuclei that enter the sporangia (Figs. 6 and 7). This network, as has been shown, develops gradually in the nuclei accompanying the breaking up of the chromatin body into irregular cloudy masses.

The chromatin network changes before mitosis into a spirem, upon which deeply-staining chromatin granules may be easily recognized. I

have seen some evidence of a centre on the exterior of the nucleus (Fig. 31), to which the spirem seemed to be attached, suggesting nuclear polarity similar to that described by Harper ('05) for *Phyllactinia.* However, the conditions in *Derbesia* are not very favourable for the study of this point, and I am not prepared to discuss the matter further at this time.

The spindle at metaphase of mitosis (Fig. 32) is intranuclear, and there is a minute granule at each pole.

Cytological Discussion.

This discussion will concern itself chiefly with the morphology and development of the blepharoplast and its relation to the nucleus. There is a wide divergence of opinion respecting these problems. Strasburger ('92, '00), from studies of zoospores, chiefly those of *Oedogonium*, *Cladophora*, and *Vaucheria*, concluded that in these forms the cilia were derived from a body (blepharoplast) which arose in the outer plasma membrane. This conclusion for *Oedogonium* is of especial interest, since the zoospore of this alga is provided with a circle of cilia similar to that of *Derbesia.* Mottier ('04) has described the blepharoplast of *Chara* as arising in the plasma membrane in agreement with Strasburger's views.

Timberlake ('02) noted fundamentally different conditions in his study of zoospore formation in *Hydrodictyon.* He described the blepharoplast as a body distinct from the plasma membrane and connected with the nucleus by delicate fibres. There is also a period during zoospore formation when the nucleus lies very close to the cleavage furrow, and at this time a granule may sometimes be observed at the side of the nucleus. Timberlake did not trace the origin of the blepharoplast, but it seems very probable that it is derived from this granule close to the nucleus, and remains connected with the latter by delicate fibres, at least during the earlier periods of zoospore formation. There is thus an apparent agreement between *Hydrodictyon* and *Derbesia* in the intimate relation of the blepharoplast to the protoplasm around the nucleus and its independence in origin of the outer plasma membrane.

The other studies on the blepharoplast of plants have been concerned chiefly with its possible relation to a centrosome. Belajeff in a series of papers ('97 *a*, '97 *b*, '97 *c*, '98, '99) described the blepharoplasts of *Gymnogramme*, *Equisetum* and *Marsilia.* He found the blepharoplasts at the poles of the spindles in the mitosis just preceding the formation of the sperms and regarded them as centrosomes. Shaw ('98) held that the blepharoplasts of *Marsilia* did not occupy the poles of the spindle of the final mitosis, but were formed at the poles of the preceding spindle; Belajeff, however, disputes this.

Ikeno ('98) for *Cycas*, Hirase ('98) for *Ginkgo*, and Webber ('01) for *Zamia* agree in describing the blepharoplasts as arising *de novo* on opposite sides of the nucleus and at some distance from it, before the mitosis that precedes the differentiation of the sperm nuclei. The spindle of this mitosis is said to hold no relation to the two blepharoplasts which are far removed from its poles. Ikeno and Hirase have from the first held that the blepharoplast was an attractive sphere or centrosome, and Ikeno ('04) has more recently emphasized his views of the homology of the centrosome and blepharoplast of plants. Webber, however, laid stress on the striking fact that the blepharoplasts are independent of the spindle fibres of the last mitosis of spermatogenesis of *Zamia*, and are formed *de novo* at some distance from this nucleus, while it is in the resting condition, a developmental history which would not be expected of a centrosome-like structure.

Ikeno ('03) has also reported centrosomes during the mitoses of spermatogenesis in *Marchantia*, stating that the centrosomes of the last mitosis become the blepharoplasts of the sperms. Miyake ('05) has not been able to confirm Ikeno's conclusions respecting the presence of centrosomes during the mitoses of spermatogenesis. However, he finds a body at each spindle pole of the last mitosis of *Marchantia* and groups of granules similarly situated in *Makinoa*, and he considers these as likely to function as blepharoplasts. Ikeno ('05) has replied to the criticisms of Miyake, but this discussion is not essential to the problem before us.

The views of botanists respecting the origin and nature of the blepharoplast are then at variance in fundamental points. Strasburger, Shaw, Webber, and Mottier hold that the blepharoplast is not a centrosome, but arises either in the plasma membrane or in the cytoplasm, independently of the nucleus. Belajeff, Ikeno, and Hirase believe it to be related to the centrosome. There are conflicting accounts of its history even in the same forms, and very little information concerning its origin even in the most favourable types, such as the cycads and *Ginkgo*.

Let us now consider the history of the blepharoplast of *Derbesia* in relation to the confusion of views described above. The blepharoplast of *Derbesia* has clearly very important physiological relations to the nucleus. The granules that enter into its composition come from the surface of the nucleus and travel along a system of protoplasmic strands to the plasma membrane *beneath which* the blepharoplast is formed. Timberlake's account of *Hydrodictyon* has many points of agreement with this history and offers substantial support, although the zoospores of that form are too small to trace the development of the blepharoplast as easily as may be done in *Derbesia*. This history for *Derbesia* does not accord at all with Strasburger's view of the origin of the blepharoplasts in *Oedogonium*, *Cladophora*, and *Vaucheria* from the plasma membrane, or the similar account of Mottier for *Chara*. I cannot but believe that a closer study of the development of the

blepharoplast in these types will show physiological connexions between this structure and the nucleus, and that the blepharoplast will be found to arise, not from the plasma membrane, but from material associated with the nucleus. The zoospore of *Oedogonium* will probably prove very favourable for investigation from this point of view.

With respect to the theory of a relationship between the blepharoplast and centrosome, *Derbesia* furnishes clear evidence in the negative. In this type the blepharoplast is formed from a very large number of granules derived from perhaps a third or a fourth of the nuclear surface, all similar to one another and to the other granules at the bases of the numerous radiating protoplasmic strands. These granules cannot be centrosomes although they may be kinoplasmic in character. I think it is possible that the centrosome theory of the blepharoplast, as held by Belajeff, Ikeno, and Hirase, will not receive support when types are studied in which the blepharoplast is not developed in close association with mitosis. The presence of a mitosis immediately preceding the formation of sperms in the liverworts and ferns and the position of the developing blepharoplast at the poles of these spindles naturally lead to its association with a centrosome. There are no mitoses immediately preceding zoospore formation in *Derbesia*, or indeed in the sporangium at all, and similar conditions are also present during zoospore formation in *Oedogonium* and a number of other Algae which should be studied. The investigation of the origin of the blepharoplast in such forms (as also in the Cycads and *Ginkgo*) will probably show that the blepharoplast, in these cases at least, is not the homologue of the centrosome as this structure is generally understood. Nevertheless, it must be borne in mind that the studies on spermatogenesis in animals have shown that the locomotor apparatus of the spermatozoid is formed in large part from the centrosome, and botanists have been fully justified in approaching the investigation of spermatogenesis in plants from that point of view. It is very important that more detailed studies on spermatogenesis in plants be undertaken upon such favourable types as *Marsilia*, certain ferns, the Cycads, and *Ginkgo*.

It is possible that both the blepharoblast and centrosome may prove to have fundamental points of resemblance in their relation to kinoplasm investing the nucleus and to systems of radiating protoplasmic strands running out into the cytoplasm. The substance of the blepharoplast and centrosome may be similar, and their position as dynamic centres may be clearly analogous, but without morphological relationship. There is much evidence that both structures are formed at the point of convergence of radiating protoplasmic strands, which at certain stages of development are likely to prove to be streams of protoplasm bringing material to a dynamic centre. When such streams converge to a well-defined centre the whole system takes the form of an aster. Indeed there are reasons for

believing that the asters associated with mitosis are such dynamic centres, and their radiating fibres either the paths of delicate protoplasmic streams or developments from such streams. According to this view the aster stands as the morphological expression of dynamic activities, and the centrosome and centrosphere are rather the result of these activities, associated with the rays converging to a common point, than the cause of the structure.

The structure of the cilia-forming apparatus in epithelial cells of animals is of interest in relation to this study of *Derbesia*. Earlier views, which held the granules below the cilia to be centrosomal in nature, have been discredited by later work. The subject is discussed in a paper of Wallengren ('05) on *Anodonta*. Wallengren has followed the mitotic division of the nucleus in the epithelial cells from the gills of this mussel. A centrosome (diplosome) is present, lying among the granules at the bases of the cilia, and accompanies the nucleus in its mitosis. During the mitosis the cilia first disappear, followed by the basal granules and the fibrils that extend from them into the cytoplasm. The cell divides by constriction after which a thick protoplasmic layer is formed under the new cuticula. Granules arise in this layer of protoplasm without genetic relation to the mitotic figure. From each of these granules a fibril is first developed extending into the cytoplasm, after which a cilium grows out through the cuticula. The granules, fibrils, and cilia form, all together, the ciliated apparatus corresponding to the blepharoplast of plants with their cilia and internal fibrils, when present.

The significant feature of this history of the development of the cilia-forming apparatus in epithelial cells, in relation to the origin of the blepharoplast in plants, is its independence of centrosomes or other structures of the mitotic figure. The fibrils penetrating the interior of the cell, however, probably hold important physiological relations to the nucleus. Such fibrils are well known in epithelial cells, and have been described and figured as penetrating to the protoplasm immediately investing the nucleus; see discussion of Gurwitsch ('04), p. 67. Their presence indicates that the granules bearing cilia have intimate dynamic connexions with the protoplasm and nucleus of the cell. The blepharoplast of *Derbesia* during its development shows clearly a similar physiological dependence upon the nucleus and cytoplasm, a dependence which probably lasts as long as the cilia are motile, although conspicuous protoplasmic strands connecting the nucleus with the blepharoplast are not evident after the blepharoplast is fully formed.

Studies upon Infusoria have established the presence of granules at the bases of the cilia, which in some forms are, of course, very numerous. Maier ('03) believes that these can hold no possible relation to centrosomes, which are unknown in the Infusoria. These conclusions are thus in accord

with those of Gurwitsch and Wallengren that the granules at the bases of cilia in epithelial cells are not related to centrosomes.

The Flagellata present excellent subjects for the investigation of cilia-forming organs, which in this group have not as yet received the attention that they deserve. Plenge ('99) established the presence of a granule at the base of the cilium in the swarm spore of the myxomycete, *Didymium*, and the connexion of this granule by a delicate fibril with the nucleus of the cell. Similar conditions were also observed by him for several flagellates. Dangeard ('01) has shown in some detail for *Polytoma* that the two cilia arise from a small body connected with the nucleus by a delicate fibril, and Maier ('03), p. 145, confirms his observations, and also reports a similar structure in the cells of *Chlamydomonas*. Prowazek ('03) studied the conditions in a number of flagellates, finding the cilium in some forms in intimate connexion with the nucleus, and in others arising from a granule connected with the nucleus by a fibril, or with its base so connected. His observations thus support those of Plenge, Dangeard, and Maier. All of these authors have discussed the possibility of the basal granule being centrosomal in character when comparing these ciliated cells with the spermatozoan, but definite conclusions on this subject must await detailed investigations on nuclear division and the origin of the cilia in daughter-cells of the flagellates. However, it does not seem probable that the basal granule is a centrosome.

There is another phase of the subject of cilia-bearing organs of animal cells which must be mentioned, since it indicates that such structures may have different origins and developmental histories in different tissues. That centrosomes are intimately concerned with the development of the locomotor apparatus of the spermatozoid seems to be established from the studies on spermatogenesis in animals; see discussions of Meves ('01) and Waldeyer ('06). There is, therefore, a situation with respect to the origin of cilia-bearing organs of animal cells apparently somewhat similar to the divergent accounts of plant cells. That is, these organs have a relation to centrosomes in the spermatozoid but an independence of centrosomes in epithelial cells, in those of Infusoria and probably also the Flagellata. Whether the blepharoplast of the plant sperm is related to the centrosome is, however, a matter demanding further investigation.

The Zoospore and Motile Gamete as Taxonomic Characters in the Siphonales.

Within recent years emphasis has been laid by a number of writers upon the zoospore and motile gamete as structures of fundamental importance in determining the relationships of great groups among the algae. The motile reproductive cells are held to retain in the various groups the

characters of a primitive ancestry from forms allied to the flagellates. In other words the periods of sporogenesis and gametogenesis are regarded as periods in the life-history when the algae return for a short time to conditions similar to those of their ancestry, and the structure of these motile cells may be expected to give important evidence of the morphology of the flagellate-like types from which various classes and orders of the algae may have been derived.

This view unquestionably has much of value both in practice and theory. However, its application may be carried to extremes, and classifications based chiefly or wholly on the zoospores and motile gametes are likely to prove artificial and open to criticism, after the manner of many systems which have been proposed on single characters. We have in the Siphonales an assemblage of algae which illustrate very clearly the danger of attaching too much importance to these reproductive cells as taxonomic characters, and *Derbesia* is of especial interest as a test case.

Blackman and Tansley ('02) in their revision of the classification of the green algae have removed *Botrydium* and *Vaucheria* from the Siphonales because the motile reproductive cells do not conform in structure to the zoospores and gametes typical of this group. Although they still retain *Derbesia* because of its general resemblance to *Bryopsis* their opinions are strongly expressed in the following remarks (p. 25 of the reprint): 'Though the vegetative characters of this genus suggest a position among the lower Siphoneae in the neighbourhood of *Bryopsis*, yet the peculiar zoospores, recalling those of *Oedogonium*, probably indicate that its affinities are widely different. Our present information is not however sufficient to justify the removal of the genus from its traditional place.'

An analysis of the process of spore formation in *Derbesia* shows at once how unsafe it would be to conclude that the peculiar structure of its zoospores has deep phylogenetic significance. The early stages of spore formation are those common to all of the Siphonales (including *Vaucheria*); the sporangium becomes filled with multinucleate protoplasm. Then in *Derbesia* there begins the peculiar process of nuclear differentiation and the degeneration of the greater number of smaller nuclei. The process of nuclear degeneration is very exceptional, although finding its analogy during oogenesis in *Vaucheria*, *Saprolegnia*, and in the Peronosporales. The result is the development of a set of extraordinarily large nuclei which become the centres of spore formation. The kinoplasmic activities concerned with these large nuclei produce blepharoplasts of remarkable form and size, capable of developing and bearing a great many cilia instead of the typical number two.

There can be little doubt that the peculiarities of the zoospore of *Derbesia* are later developments in phylogeny concerned with the exceptional features of the process of sporogenesis, and that its structure is not

that of ascestral forms and can have no value in phylogenetic considerations. The evidence indicates that the ancestors of *Derbesia* produced immense numbers of small zoospores represented by the very numerous nuclei which enter the sporangium, and it is very probable that these zoospores were similar to the biciliate elements characteristic of the *Siphonales*.

The zoospore of *Oedogonium* has a structure similar to that of *Derbesia* with respect to the arrangement of its cilia, and I venture to predict that the development of its blepharoplast will be found to parallel closely that of *Derbesia*, for the reason that the zoospore is developed from an exceptionally large uninucleate protoplast. However, I hardly think that any one for these reasons will be bold enough to suggest a relationship between *Derbesia* and *Oedogonium* in view of the striking differences at all points in their general morphology.

Derbesia may serve as a warning of the danger of carrying too far the practice of basing classifications of the algae on the structure of the zoospores and motile gametes. The removal by some authors of *Vaucheria* from the *Siphonales* seems to me open to serious criticism, and has not been followed by other authors, as, for example, Oltmanns ('04). *Botrydium* is of course a peculiar type of uncertain position. This study of *Derbesia* shows clearly the necessity of treating exceptional forms of algae separately, deferring judgement on the significance of peculiarities of zoospores and motile gametes until their development and structure is understood in comparison with these elements in groups of supposed relationship.

Summary of the Investigation of Derbesia.

The protoplasm in the globular sporangium becomes separated from that of the parent filament by the formation of a heavy cellulose plate, which begins to develop as a ring at the periphery, and consists of layers deposited successively as the protoplasm in the sporangium and filament withdraw from one another.

The protoplasm within the developing sporangium contains several thousand nuclei. These are all similar to one another, and slightly larger than the plastids at the time when the cross-partition is formed at the base of the sporangium. Each nucleus contains a small nucleolus and a large chromatin body.

A process of nuclear differentiation sets in very shortly after the protoplasm of the sporangium becomes separated from that of the parent filament. Certain nuclei increase in size, reaching a diameter 4–6 times that of the plastids. These larger nuclei also become conspicuous because of the development of numerous protoplasmic strands which radiate out from the cytoplasm enveloping the nucleus. The strands have deeply-staining granules where they join the enveloping cytoplasm just outside of the

nuclear membrane. The larger nuclei become distributed rather uniformly throughout the sporangium, lying generally at some distance from one another.

The smaller nuclei are found sometimes singly but more frequently in groups within areas of cytoplasm bordered by plastids. They never fuse with one another. These smaller nuclei gradually decrease in size, becoming much smaller than the plastids, and finally, losing their chromatin content, break down in the cytoplasm. They have generally disappeared completely at the time the spores are formed, except for deeply-staining globules in the cytoplasm which are the remains of the nucleoli.

The large surviving nuclei are not associated with cytoplasmic centres such as coenocentra, but the arrangement of the protoplasmic strands indicates that they are the paths of cytoplasmic streams passing to and fro between the nuclei and the cytoplasm. The large nuclei are, therefore, probably themselves important centres of dynamic and metabolic activity.

The segmentation of the protoplasm does not begin until the process of nuclear degeneration is practically ended. Cleavage begins at the periphery of the protoplast and the protoplasm is quickly cut by curved and branching furrows, that finally divide it into uninucleate masses, which are the zoospore-origins. Occasional binucleate spore-origins have been noted. The zoospore-origins round up and the plastids take a radiate arrangement around the centrally placed nucleus with its investing envelope of cytoplasm and radiating protoplasmic strands.

The nucleus of the spore-origin then moves from the centre of the protoplast towards the periphery. Perhaps a third or a fourth of the protoplasmic strands on the side of the nucleus nearest the periphery become arranged in the form of a funnel. Granules may be found on these strands apparently moving outward towards the plasma membrane.

These numerous granules accumulate in a circle just underneath the plasma membrane and fuse with one another to form a deeply-staining, firm ring, which is the blepharoplast. The strands connecting the nucleus with the developing blepharoplast apparently disappear after its development, and the nucleus passes back to the centre of the zoospore, the plastids becoming distributed about it again in a radiating arrangement.

The blepharoplast then splits to form two rings, one slightly below the other, and the circle of cilia is developed from the lower ring.

During the development of the zoospores the homogeneous chromatin body undergoes changes; its material becomes granular or cloudy and strands appear, forming an irregular network of coiled threads, from which is developed the spirem of the first mitosis in the germinating spore.

The two rings of the blepharoplast remain for some time closely pressed against the cell wall at the base of the germinating spore, but they

gradually grow fainter and probably at last disappear. The nucleus of the germinating spore divides mitotically, the spindle being intranuclear.

It is clear that the nucleus of *Derbesia* has very intimate relations to the development of the blepharoplast, and that the latter does not arise from the plasma membrane, but from granules associated with the protoplasm investing the nucleus. These granules are not, however, centrosomes.

CAMBRIDGE, U.S.A.,
March, 1907.

Note:—Since the above was written there has appeared a paper by Escoyez ('07), 'Blépharoplaste et Centrosome dans le *Marchantia polymorpha*,' La Cellule, xxiv, 247. This author, contrary to Ikeno ('03), finds no centrosomes in the mitoses preceding the differentiation of the sperm-mother-cells. Blepharoplasts, two in number, were first observed in contact with the plasma-membrane in the sperm-mother-cell and they occupy the poles of the spindle of the mitosis within this cell, which precedes the organization of the two sperm-nuclei. Escoyez does not consider the blepharoplast to be a true centrosome. He has not traced its origin, and we do not know whether it comes from within the cytoplasm near the nucleus or is formed in the plasma membrane. The former alternative is to be expected.

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EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Dr. Davis's paper on *Derbesia*.

The preparations were studied under the Zeiss apochromatic oil immersion 1·5 mm. with the compensating oculars. The figures were sketched with an Abbé camera from sections generally cut 3 μ thick and stained with iron-alum haematoxylin after the method of Heidenhain. The magnification was as follows: Fig. 1, 1000 diameters; Figs. 2, 12, 13, and 27, 667 diameters; Figs. 6-9, 14-26, 28, and 29, 1334 diameters; Figs. 10, 11, and 30–32, 2000 diameters.

PLATE I.

Fig. 1. Zoospore in side view, stained with iodine to show circle of cilia.

Fig. 2. Zoospore shortly after coming to rest, viewed from above, stained with iodine.

Fig. 3. Outline of a sporangium in section, the protoplasm of which is still in communication with that of the parent filament.

Fig. 4. A stage in the formation of the cross partition at the base of the sporangium; there is present a ring-shaped thickening growing inward from the periphery and showing a laminate structure.

Fig. 5. The cross partition at the base of a sporangium showing the laminate structure.

Fig. 6. The nuclei as they enter the sporangium, illustrating their relative size in comparison with the plastids; each nucleus contains a small nucleolus and a large chromatin body.

Fig. 7. Large and small nuclei within a young sporangium before the cleavage of the protoplasm.

Fig. 8. A large nucleus heavily stained, showing the enveloping cytoplasm and radiating protoplasmic strands.

Fig. 9. A large nucleus lightly stained to differentiate the granule at the base of each protoplasmic strand.

Fig. 10. A large nucleus and a neighbouring group of small degenerating nuclei in a region of cytoplasm free from plastids; the latter are now much smaller than the plastids.

Fig. 11. A group of smaller nuclei in an advanced state of degeneration; the nuclear membranes of some have disappeared, and the nucleoli lie in the cytoplasm.

Fig. 12. The beginning of segmentation by branching cleavage furrows cutting into the protoplasm from the periphery.

Fig. 13. Spore-origins rounding up.

Fig. 14. A spore-origin, showing the centrally placed nucleus from which protoplasmic strands radiate among the plastids, whose long diameters generally point towards the nucleus.

Fig. 15. A nucleus lying near the periphery of a spore-origin, the granules apparently passing outward along the protoplasmic strands between the nucleus and the plasma membrane.

Fig. 16. A stage similar to, but slightly older than that shown in Fig. 15. The nucleus and funnel-shaped arrangement of the protoplasmic strands with the granules is viewed from the interior of the spore-origin.

Fig. 17. The granules are arranged in a circle just underneath the plasma membrane.

PLATE II.

Fig. 18. Portion of a circle of granules underneath the plasma membrane, viewed from the exterior of the spore-origin.

Fig. 19. The granules have fused to form a firm ring, the blepharoplast, above the nucleus and just beneath the plasma membrane.

Fig. 20. A spore-origin after the formation and splitting of the blepharoplast ring, showing the nucleus now in the centre of the protoplast and the plastids again arranged in a radiating manner.

Fig. 21. The blepharoplast (*b*) in section before it splits to form two rings. A chromatin network is clearly present in the nucleus.

Fig. 22. The mature blepharoplast, showing cilia developing from the lower of the two rings.

Fig. 23. The splitting of the blepharoplast (*b*), as seen in section, to form two rings.

Fig. 24. A portion of a blepharoplast, showing the two rings.

Fig. 25. The two rings of the blepharoplast, seen in section, with cilia attached to the lower.

Fig. 26. The blepharoplast in section, cilia attached to the lower ring on the right-hand side.

Fig. 27. A spore-origin with two nuclei and a portion of the blepharoplast; this would have developed into a binucleate zoospore.

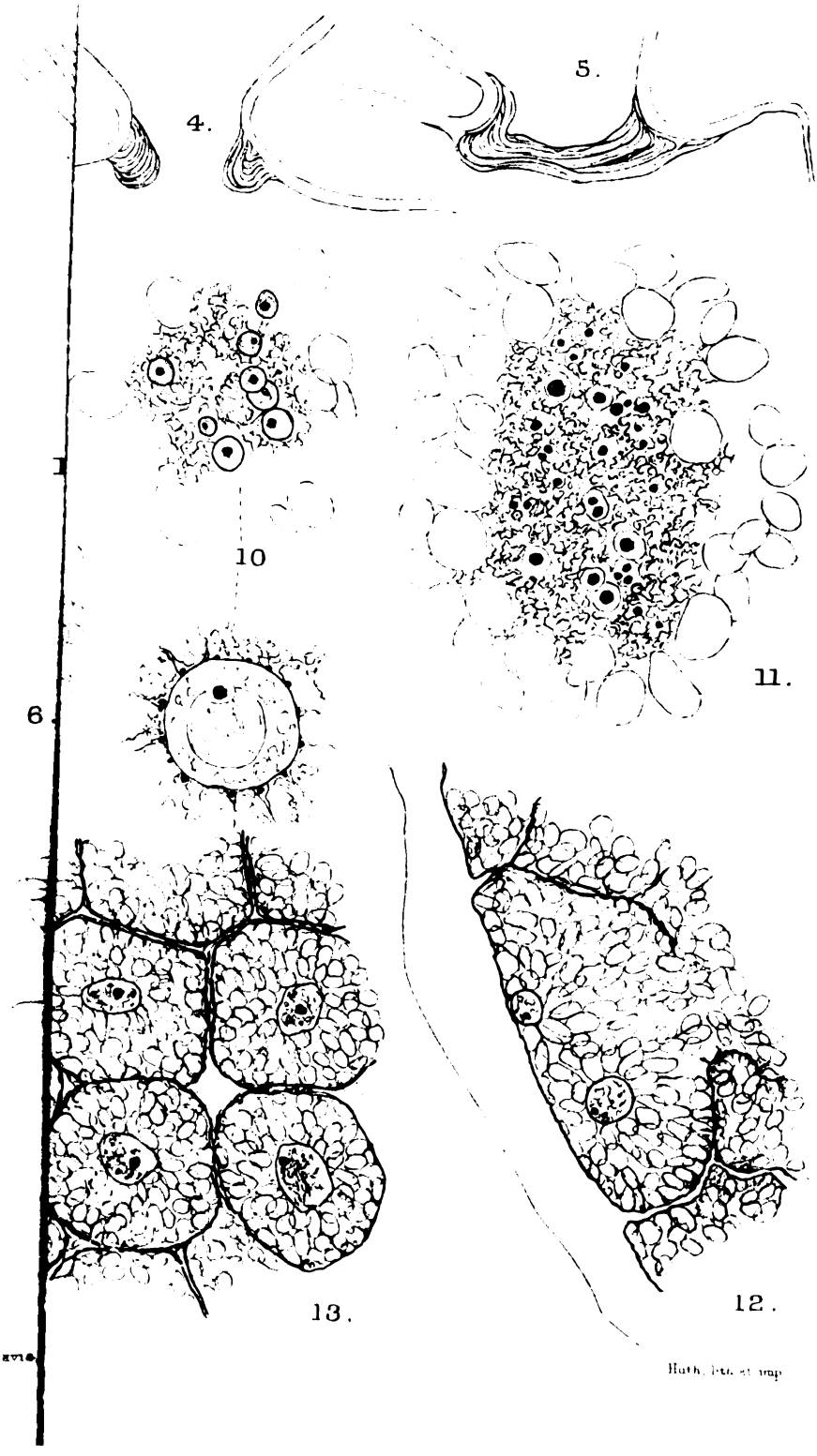
Fig. 28. Base of uninucleate germinating spore, showing remains of blepharoplast (*b*).

Fig. 29. Base of sporeling containing several nuclei; the rings of the blepharoplast (*b*) still present.

Fig. 30. Nucleus in germinating spore, showing loose linin network with chromatin granules.

Fig. 31. Nucleus in germinating spore, showing a possible much-coiled spirem apparently connected with a centrosome-like body on the exterior of the nucleus.

Fig. 32. Metaphase of mitosis in the sporeling, an intranuclear spindle.



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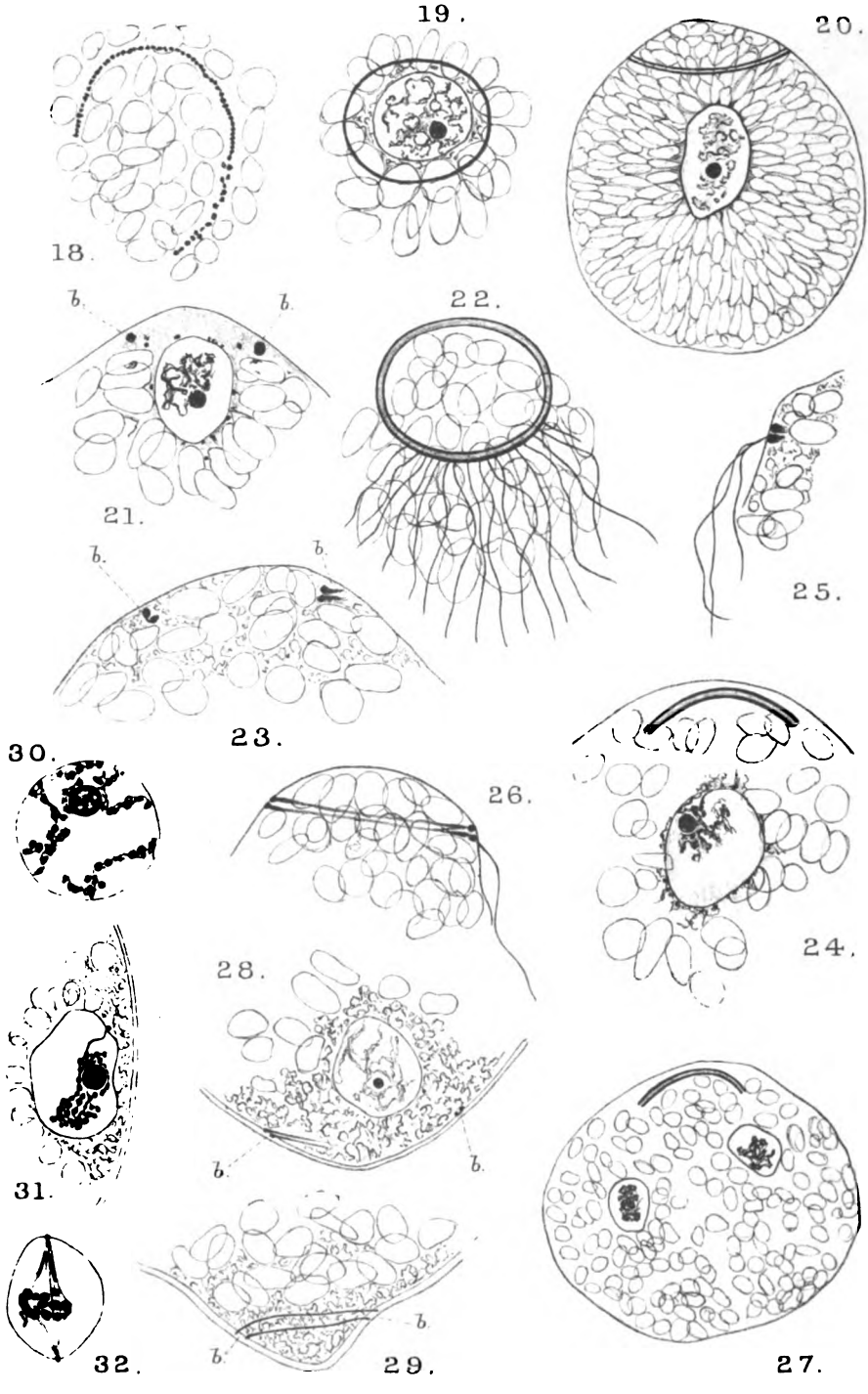

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DAVIS — DERBESIA

The Origin of the Roots in Lycopodium Selago.

BY

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With Plate III.

THE question of the origin of the roots of Lycopodiaceae has received the most detailed discussion from Bruchmann[1, 2] and from Van Tieghem,[3] both of whom worked especially at *Lycopodium inundatum*. They arrived at different conclusions, and it was suggested to me that an examination of the root origin in some other species of *Lycopodium* might throw some light on the subject. My observations of *L. Selago*, however, incline me to think that the process in this species differs from that described by either Bruchmann or Van Tieghem, although Van Tieghem[4] states that *L. Selago* agrees with *L. inundatum* in all but the course followed by the root through the cortex.

I have chosen for investigation *L. Selago*, since this species differs considerably in habit from *L. inundatum*, and does not appear to have been so carefully studied in this connexion as has the latter species. *L. Selago* is of particular interest, since it is an upright form of *Lycopodium*, and the roots arising near the apex pass down through the cortex of the stem, each root surrounded by its own cortical tissue (see Pl. III, Fig. 7).

In both papers by Bruchmann it is stated that in *L. inundatum* the root arises from several layers of the inner cortex, the plerome periblem and dermatogen of the root all being formed from the cortex of the stem. The pericycle, which he prefers to call pericambium, at a later stage helps to connect the base of the root on to the stem. He calls the layer of cells adjacent to the primary tracheids of the stem the pericambium, and he considers that this layer is formed from the periblem of the stem.

[1] Bruchmann (*a*), Über Anlage und Wachstum der Wurzeln von *Lycopodium* und *Isoëtes*. Jen. Zeit. für Med. u. Nat., Bd. viii, 1874.

[2] Bruchmann (*b*), Über Prothallien u. Keimpflanzen mehrer europäischer Lycopodien, 1898.

[3] Van Tieghem et Douliot, L'origine des membres endogènes dans les plantes vasculaires. Ann. des Sc. Nat., 7e série, tome viii, 1888.

[4] Van Tieghem, loc. cit., p. 557.

He states that the roots arise in cells outside the layer next the tracheids, before the endodermis is differentiated from the pericambium.[1]

Van Tieghem,[2] on the contrary, asserts that the whole root is formed from the true pericycle, which consists of one layer of cells, the outermost layer of the plerome of the stem. In this point he finds that the Lycopodiaceae agree with all the Phanerogams and with Isoëtes, and differ from all other Cryptogams, in which the roots are formed from the endodermis, or in the case of *Selaginella* and *Equisetum*, from layers still farther outwards from the plerome. He does not state how he distinguishes this pericylic layer in *L. inundatum* from the endodermis before the cell-walls of the latter have become thickened, and Bruchmann, in arguing against Van Tieghem's theory of pericyclic origin of the root, refers to the impossibility of distinguishing the pericambium in the still meristematic part of the stem. Van Tieghem advances, as proof of the pericyclic origin of the root, the occurrence of the endodermis, with thickened walls, passing completely round the apex of the root at a stage when the three meristematic regions of the root are differentiated, and he assumes that the layer within this endodermis is the true pericycle, formed from the stem plerome.

Bruchmann in a figure of a section of *L. clavatum*, at a stage corresponding to that of Van Tieghem's figure of *L. inundatum*, marks the endodermis stopping short at the sides of a root base, and apparently forming the part of the latter nearest to the stem plerome.[3, 4] A section like that of Bruchmann might be obtained by cutting the stem at a point where the root has broken through the endodermis and is growing outwards through the cortex. When the root has reached this stage with numerous layers of cells, the exact part played by a layer of cells of the stem in the production of the root can scarcely be proved from the evidence of continuity of cell-layers.

Both authors worked chiefly at the same species, namely *L. inundatum*, which seems to differ from *L. Selago*, firstly in the fact that, in the latter, the plerome of the root is formed entirely from that of the stem, and is not formed from the same initial layer of cells as the periblem. In the latter characteristic it would therefore seem to correspond more nearly to Lemaire's fourth type of root origin.[5] Lemaire worked at the origin of lateral roots in Dicotyledons, and came to the conclusion that although as a general rule roots arise from the pericycle of the stem, which forms all the tissues of the root, other methods of root origin do exist. For example, in the case of *Asperula odorata* the central cylinder of the

[1] Bruchmann (*b*), loc. cit., p. 75 and following.
[2] Van Tieghem, loc. cit., p. 561.
[3] Bruchmann (*b*), loc. cit., Fig. 32, Taf. iv.
[4] Van Tieghem, loc. cit., Fig. 582, Pl. xl.
[5] Lemaire, Recherches sur l'origine et le développement des racines latérales chez les Dicotylédones. Ann. des Sc. Nat., 7e série, tome iii, 1886, p. 175.

root is not formed from the pericycle, but from a deeper layer, whilst the cortex and cap are alone developed from the pericycle.[1] Van Tieghem regards this simply as a case of retarded root formation, and differs from Lemaire, amongst other points, in attributing the formation of *all* the tissues of the root in *A. odorata* to layers within the pericycle.

The Origin of the Roots.

The earliest stages of root origin in *L. Selago* were found by cutting transverse and vertical sections through the upper end of the shoots of young plants grown from bulbils, at a stage when the stem bore only a few leaves (from 3 to 10). The chief difficulty in obtaining the very early stages lies in the fact that the roots arise near the apex of the stem, below the origin of several leaf-traces, and often on a level with others. In this region all the tissues of the stem are in a meristematic condition, and the central cylinder is not clearly differentiated from the surrounding cortex. The beginnings of the leaf-traces might therefore be readily mistaken for those of roots. A little lower down the stem, the innermost layers of the cortex are dividing to form the endodermis and the so-called pericycle, and possibly Bruchmann, who attributes the root origin in *L. inundatum* to a few cells in these layers, has mistaken these divisions for those of root origin.

In the earliest stages of what are undoubtedly roots, all the cells taking part in root formation are characterized by their large size and dense protoplasmic contents, and the region of cells affected includes nearly half of the central cylinder of the stem. One of the youngest roots of which I obtained a section is shown in Fig. 1, arising from the stem, which is here cut transversely. The central plerome of the stem is of small cells, none of which are differentiated into xylem or phloem. Surrounding this is a ring of cells (*i*) varying in size and intermediate between the small inner cells of the plerome (*pl*) and the large cells of the cortex (*c*). The innermost row of cortical cells has divided to form two or three rows of inner cortex, and in some parts the intermediate cells appear continuous with the radial rows of cells formed in this way. The intermediate cells therefore I consider are derived from the periblem, and probably at a later stage form the characteristic endodermis. The section figured is through the centre of the root origin, that is to say, it is the section showing the root origin at its widest point. No other sections of the series show cells farther out in the inner cortex having the dense protoplasmic contents characteristic of the root origin. It is seen from the figure that the outermost layer of cells of the root origin is continuous with the intermediate layer of cells, which cannot be defined with certainty either as pericycle or endodermis, though apparently derived from the periblem. In other

[1] Lemaire, loc. cit., p. 234.

sections (see Fig. 2) this row of intermediate cells is seen to be directly outside the first xylem elements (*v*) which are differentiated in the stem plerome, and would therefore correspond in position to the pericambium of Bruchmann.

In central transverse sections of a slightly older root origin, the inner and radial walls of the cells of the innermost layer of the stem cortex stained slightly with fuchsin-ammonia, though not with cotton red, iodine green, or aniline-water safranin (see Fig. 3). At this stage the endodermis appears continuous with the outermost layer of the root origin, but this appearance would be produced if the root formed in an inner layer had begun to grow out, and the pressure or secretions from the root from one side of the stem plerome had in some way hindered thickening of the endodermal cell-walls in that region. The cortical cells immediately outside the root origin are preserved in places, and show no thickening of cell-walls. Those around the apex of the root are not well preserved in these sections, owing to the absorptive nature of the outermost layers of the root. Fig. 4 shows a transverse section near the apex of the stem of an older plant. The stem is cut above the last point of dichotomy, through the part bearing sporangia. The section shows the root base surrounded by mucilage when only slightly projecting from the circumference of the stem stele, so that even if the stem endodermis originally surrounded the root base, as Van Tieghem figures for *L. inundatum*, in *L. Selago* it would be disorganized by the root-cap before the root grew out as far as that figured by Van Tieghem. This agrees with Bruchmann's statement for *L. inundatum*, that the endodermis of the stem is not seen covering the root base; Bruchmann, however, as stated above, found the endodermal cells—with darkly staining walls—passing up to the centre of the sides of the root base in a vertical section. He uses as an argument in favour of the cortical origin of the root the fact that the cortex and the epidermis of the root pass laterally into the cortex of the stem, not reaching the vascular cylinder of the latter, and also only the few outer layers of the stem cortex are destroyed by the outward passage of the root.[1] These points he says are noticeable, not only in *L. inundatum*, but also in *L. clavatum*, *L. annotinum*, *L. complanatum*, and *L. alpinum*.[2] That this is not the case in *L. Selago* is seen from Fig. 5, where the periblem of the root is continuous with the plerome of the stem, and also from Fig. 4, where the stem cortex is destroyed round the root up to the point where the latter begins to curve out from the stem plerome.

From the study of such sections it seems clear that the outermost layer of the young root is derived from an intermediate layer which, as stated above, corresponds probably to the endodermis. The layer of the plerome of the stem lying within this gives rise to one meristematic layer

[1] Bruchmann (*b*), loc cit., p. 77. [2] Ibid., p. 80.

which divides tangentially to produce the periblem of the root, which usually consists, even in the young root, of two or three layers of cells at the apex (Figs. 1 and 2). It cannot be said with certainty which layer of undifferentiated tissue of the stem first produces this meristem, but it is certainly produced from a portion of the plerome. The plerome of the root is also directly derived from that of the stem, but the root arises at a stage when the vascular elements of the stem are undifferentiated. In Fig. 4 the xylem masses of the stem are seen not yet united in the centre of the stele, and all the xylem cells are not yet lignified. The centre of the root base is seen to be connected with the phloem, though the two xylem groups of the stem, one on either side of the root base, have not yet become connected with the root xylem. All the tissues of the root are still in a meristematic condition, but the apex, showing the three distinct meristematic regions, is found several sections (about 250 μ) lower down the stem, lying completely in the stem cortex.

Bruchmann [1] states that the tracheids in *L. inundatum* only appear at the base of the root when the latter breaks through to the outside, but in *L. Selago*, owing to the longer period which the root passes in the stem cortex, the xylem is differentiated before this point is reached. Jones [2] (in describing *L. Selago*) as well as Bruchmann [3] (referring to *L. clavatum* and *L. inundatum*) agree that the phloem of the root is directly connected with that of the stem, and Van Tieghem [4] states that the root of *L. inundatum* is formed in the pericycle opposite the place where a phloem bundle will form later.

The above conclusions are drawn from a study of transverse sections of stems at varying ages. Vertical sections near the apex of the stem show these points less clearly, since such different conclusions may be drawn from sections which are slightly oblique or not exactly median, and also since the stem endodermis does not stain except where the roots have begun to dissolve the surrounding tissues.

Fig. 5 represents a vertical section of a young shoot grown from the bulbil, and shows a young root origin. The dermatogen of the root, of one layer of cells, appears continuous with the innermost layer of wide cortical cells. The periblem and plerome of the root are continuous with several rows of cells of the stem plerome. The enlarged cells forming the base of the root plerome are seen to come into contact with one of the annular vessels of the stem, but no vessels are formed as yet in the root itself. The plerome cells of the stem are distinguished by their narrow elongate form, and elongate darkly-staining nuclei; in some sections round or oval sieve-

[1] Bruchmann (*b*), loc. cit., p. 81.

[2] Jones, Morphology and anatomy of the stem of Lycopodium. Trans. of Linn. Society, 2nd ser., Botany, vol. vii, Pt. II, 1905, p. 29.

[3] Bruchmann (*b*), loc. cit., p. 79 &c.; Fig. 31, Taf. iv.

[4] Van Tieghem, loc. cit., p. 556.

plates were found on the walls of such cells. These cells become shorter and rounded in the region of the root base, and the nuclei also become rounded. In this and other vertical sections the outermost layer of these cells is seen to be continuous with the periblem of the root base, i. e. the second layer from the outside of the base. The dermatogen is continuous with the innermost layer of cells which are without the narrow elongate nuclei, and since these cells are also wider, it may be concluded they are cortical in origin.

The Growth of the Roots.

Bruchmann favours the view that there are four distinct histogenous layers in the roots of Lycopodiaceae, while Van Tieghem is of the opinion that there are only three. Van Tieghem in this point follows Strasburger. The answer to the question depends on the method of formation of the root-cap. Van Tieghem [1] states that the cap is formed from the epidermis, which may become from twelve to fifteen layers thick. Strasburger [2] is of the opinion that the calyptrogen is formed in the first place from the dermatogen, but at once begins to divide tangentially and radially, independently from the latter. Bruchmann, on the contrary, in his earlier work, in which he enters more especially into the question of root origin, and again in his second treatise, says that formation of the root-cap by tangential division of the dermatogen seldom occurs, but in most cases an independent calyptrogen is formed.[3] In *L. Selago* I find the dermatogen soon divides tangentially two or three times, the first divisions being at the sides, not in the centre of the apex. The cells cut off on the outside form the root-cap seen in all but the youngest roots. These cells digest and absorb the surrounding cells of the cortex, leaving a way clear for the root to pass through. In vertical sections through the root apex, as for example in Fig. 6, the cap is seen to consist of three or four rows of cells arranged in radial lines corresponding with dermatogen cells. The cells of each row increase in size from the periblem to the apex of the root-cap. This seems to me to be evidence in favour of the view that there is no definite calyptrogen, that is, that there are only three meristematic layers in the root. At a later stage than that represented in Fig. 5, several of the cells in the region of the apex divide together in the periblem, at first tangentially, forming two or three layers of cells, then radially. As many as four rows of periblem cells were seen at the centre of the apex in some roots still in the cortex. The cells of the plerome, on the contrary, divide

[1] Van Tieghem, loc. cit., p. 554–5.

[2] Strasburger (*a*), Das Bot. Pract., 2nd ed., 1887, pp. 59, 60; and (*b*) Conif. u. Gnet., 1. Aufl., Jena, 1872, p. 355, Fig. 32, Taf. xxv.

[3] Bruchmann (*b*), loc. cit., p. 71–2. See also for further references Nägeli u. Leitgeb, and Reinke.

first radially, forming several radial rows of cells, the number varying from about three to six, then tangentially causing outward growth of the plerome. Since, however, the periblem curves round the plerome, the radial divisions of the former are in the same direction as the tangential divisions of the latter. My observations thus agree with Bruchmann's conclusions, stated in his earlier work, that the roots grow without apical cells.

The Course of the Roots through the Cortex.

L. Selago differs from *L. inundatum* in the point at which the roots arise. According to Bruchmann's first treatise, the roots of the latter species appear at the top of the stem before the youngest leaves, seldom later as in other Lycopods. In his later work,[1] however, he says they sometimes begin to form before the youngest leaf-trace arises, but mostly afterwards. Jones[2] merely states that the roots of *L. Selago* arise quite high up the stem, even above the point where the latter has branched, while Strasburger[3] finds internal roots of the same species usually appearing above the first dichotomy. I find roots arise in this species only below the point of origin of several leaf-traces. In an old plant they were found still at a fairly young stage, near the apex of the stem which bore sporophylls, though below the youngest leaves.

With reference to the course taken by the roots through the cortex in *L. Selago*, Bruchmann[4] states that the root grows downwards in the plant, parallel to the vascular cylinder, and, as soon as it finds a suitable position, it breaks through the remaining layers of the cortex and passes out into the ground. Van Tieghem[5] mentions that the root, after passing vertically down through the cortex, escapes at the level of the ground. I find in the same species the roots arising at the apex of an upright stem pass first obliquely, then directly down, through the middle cortex until the surface of the soil is reached and a bend in the stems occurs. No roots appear outside the stem above this point. Numerous roots may come off from the stem stele below the highest point at which a root breaks through to the outside.

It was stated above that the roots are each connected with one phloem group: the two xylem groups adjacent to the same become connected with the root xylem at a later stage. The roots, however, do not arise from each phloem group in a definite order. For instance, if we name the phloem groups of the stem I, II, III, then in one series of transverse sections down the stem it was seen that the roots arise from I, then II, then I again, not

[1] Bruchmann (*b*), loc. cit., p. 75.
[2] Jones, loc. cit., p. 22.
[3] Strasburger (*c*), Eine Bemerkung über Lycopodiaceen. Bot. Zeit., 1873, p. 109.
[4] Bruchmann (*b*), loc. cit., p. 101.
[5] Van Tieghem, loc. cit., p. 557.

in connexion with each phloem group in turn, I, II, III, I, &c. Where the stem branches the roots pass from the cortex of the smaller branch into the cortex of the main branch, and the bases of roots were seen close to the point of dichotomy of the vascular strand of the stem, showing that the roots arise from each of the two vascular strands while the latter are still enclosed in one cortex. It was found also, that where the stem has grown obliquely to the ground plane, the roots are all in one half of the cortex; where the stem grows upright, the roots are arranged in the cortex on all sides of the stele; Fig. 7 shows eleven roots lying almost entirely in one half of the cortex of the stem. A section about a centimetre higher up the same stem showed roots all round the stele, but between the two points of section the stem had curved upwards. The lower section, i.e. the one figured, is cut from a part of the stem just above the ground, above the highest point at which a root appears outside the stem. In one case of a young plant from a bulbil, the second root, coming from the part of the stem surrounded by bulbil leaves, was seen to run transversely across the stem and turn slightly upwards, so that in a transverse section of the stem the meristematic apex of the root was cut vertically. Here, however, the bulbil was fixed in the ground with its base pointing obliquely upwards. These facts I consider prove that the course followed by the roots through the cortex depends upon the position of the stem relative to the ground. Bruchmann [1] in his earlier paper mentions that the course of a root relative to the stem is altered where the latter undergoes gradual rotation, but he states that moisture and darkness appear to be the only factors which cause this change. I should ascribe it, in all the examples of *L. Selago* mentioned above, rather to the force of gravity, but the other conditions referred to may play some part, since the roots only appear on a level with the soil. Jones [2] mentions the fact that the roots only emerge where the stem is growing obliquely, but does not enter into detail, or give his conclusions as to its cause.

A large proportion of the roots present in any stem are found with their meristematic apices still embedded in the cortex of the latter. Strasburger [3] states that the roots often dichotomize before leaving the cortex of the stem, but I have not observed this in *L. Selago*, although one root was seen dividing when only two-thirds free from the cortex. For *L. dichotomum*, however, Strasburger's statement holds good, and two root bundles may often be seen enclosed in one cortical sheath.

The lowest root in the young plant grown from the bulbil, that is the first formed root, from the outside appears to be directly continuous with the central strand of the bulbil. Strasburger,[4] however, refers to the fact that in reality it arises from the side of the stele and at once directs itself

[1] Bruchmann (*a*), loc. cit., p. 527 and following.
[2] Jones, loc. cit., p. 22.
[3] Strasburger (*c*), loc. cit., p. 110.
[4] Strasburger (*c*), loc. cit., p. 114.

obliquely downwards. It becomes surrounded with its own cortex, and reaching the base of the bulbil, it passes to the outside, curving round until appearing to come from the centre of the base. The stem bundle persists as a simple structure like a leaf-trace, passing actually through the lower epidermis of the bulbil, and broken short at the point where the bulbil separated from the stem. This first root bundle lies between the concave surface of the bulbil and the stem stele (see Fig. 8). Cramer [1] showed that the first root arose while the bulbil was still attached to the parent plant; but he said it arose at the point of separation of the bulbil. A vertical section, in the plane passing from concave to convex side of a bulbil taken from the stem, shows the first root with the meristematic apex still in the cortex of the bulbil shoot. Such sections through the largest bulbils found on the stem show at the same time a second root close to the apex of the stem of the bulbil, on the same side of the stele as the first root, but below two or three leaf-traces. Bruchmann [2] worked at the young sporophytes of *L. Selago* grown from prothallia, and found that the first root was continuous with the central vascular strand of the stem.

The Structure of the Roots.

As I have already stated, the old root is continuous at its base with two protoxylem groups of the stem and the intervening phloem. Bruchmann [3] and Jones [4] both found this to be the case in the species they examined, and Bruchmann and Van Tieghem [5] agree in saying that the roots arise opposite the phloem groups. Working at *L. Selago* I noticed sections of one specimen in which the root was connected with three protoxylem groups; and Miss Wigglesworth [6] figures a similar case for the first root of *L. complanatum*. In no section of *L. Selago* was a root seen to be connected with only one xylem group. The leaf-traces, on the contrary, never connect with more than one group of protoxylem cells. This difference between the root and leaf-traces is noticeable in all stems in which the xylem and phloem are differentiated. In the region of origin of the roots, however, the cells of the stem plerome all appear alike; but even here the leaf-trace origin and root origin can easily be distinguished by the amount of stem plerome affected in each case. During root origin half the plerome or more consists of enlarged cells with dense protoplasmic contents and large, darkly-staining nuclei, whilst only a few cells of the plerome are differentiated for the origin of a leaf-trace, and even these are not especially

[1] Cramer, Pflanzenphys. Untersuch. v. C. Nägeli u. C. Cramer, 1855, vol. iii, p. 19.

[2] Bruchmann (*b*), loc. cit., p. 101; also Fig. 42 u. 43, Taf. vii.

[3] Bruchmann (*b*), loc. cit., p. 81.

[4] Jones, loc. cit., p. 28.

[5] Van Tieghem, loc. cit., p. 556.

[6] Wigglesworth, Young Sporophytes of *L. complanatum* and *L. clavatum*. Annals of Botany, vol. xxi, No. lxxxii, April, 1907, Fig. 1, p. 224.

enlarged. Fig. 2 shows a root origin and base of a leaf-trace, at the same level in the stem, both being cut transversely.

The vascular bundle in an older root is usually seen in transverse sections to consist of either one continuous xylem group, in the shape of a horse-shoe, with protoxylem at the ends of the two arms, and metaxylem in the centre, or else to consist of two separate parallel bands of xylem. In the latter case there are four protoxylem groups, one at each end of each band of xylem. Both types are shown in Fig. 7, and the first type is seen in more detail in Fig. 10. The root is so orientated that the line between the two xylem groups in the latter case, or through the centre of the horse-shoe in the former, is radial in the stem cortex. The concave side of the horse-shoe is always turned away from the stem (see Fig. 7). Jones[1] finds that a very large percentage of the roots are tetrarch, but that the diarch type occurs fairly commonly also. Bruchmann,[2] on the other hand, describes the type with two separate xylem bands as that typical of most roots, and he finds that only the first and second embryonic roots, and the root from the hypocotyl, have one group of tracheids and one of phloem cells. My observations agree more with those of Jones, in that I find most roots with diarch xylem, but I also find a close connexion between the two types. At the highest level at which the root xylem is seen separate from the stem xylem in any particular root, the xylem of the root may be in one group or in two. An intermediate stage may also be seen in which the xylem is still horse-shoe shaped, but the part opposite to the open end of the horse-shoe is composed of protoxylem cells, and by slight separation in this region the tetrarch type would be produced (see Fig. 11). This intermediate type is therefore really triarch, but I find no triarch type in which the xylem is drawn out into three rays. Lastly, in some roots scattered protoxylem elements are found round the outer side of the metaxylem bands (cf. Figs. 9 and 10). The type lower down the root is not entirely dependent on that at the base. A diarch root may become tetrarch or vice versa, though more often the same type is retained throughout the root. Even close to the meristematic apex, where the xylem cells can be distinguished by their size and position, though no lignification has yet taken place, the root may be either tetrarch or diarch. On the average, more roots show the diarch structure. Of those breaking through to the outside I find all diarch, none tetrarch. That the diarch type is more usual may be gathered from the fact that out of 25 roots in one stem, traced from the base of the root to the apex (or point of breaking out from stem cortex), 17 were diarch, 3 were tetrarch, throughout;—4 were first tetrarch then diarch, and 1 was first diarch (or rather intermediate) then tetrarch. The protoxylem cells are distinguished from the metaxylem by their smaller size, but more especially by their earlier

[1] Jones, loc. cit., p. 29.

[2] Bruchmann (*b*), loc. cit., p. 81, 102.

lignification; they are lignified nearer the tip of the root, i.e. before the metaxylem becomes lignified, and are therefore seen clearly after staining with cotton red, &c. Figs. 9 and 10 are sections cut near the tip of the roots showing the difference between the metaxylem and protoxylem.

In vertical sections the protoxylem is seen to consist of narrow spiral and annular vessels; while the metaxylem is composed of wide tracheids, either scalariform, or with several rows of pits. The phloem shows oval plates with pits, on the walls of the elongate cells which form the sieve-tubes.

Outside the vascular bundle of each root is the endodermis, usually dividing into two or three layers of cells, the inner and radial walls of the inner cells being thickened. The endodermis of an old root is continuous with that of the stem. It is surrounded by a wider ring of lacunar tissue. The cells separate at an early stage in the formation of the periblem, leaving large lacunae; it is therefore difficult to obtain sections in which this tissue is well preserved. In *L. Selago* the middle or lacunar cortex of the root is continuous with the inner cortex of the stem.

The outer cortex of the root is of cells with thickened cellulose walls (see Fig. 12). Each root lying in the cortex of the stem appears to be surrounded by a layer of mucilage, formed by the partial decomposition of the cortical cells of the stem by the absorptive root-cap.

The structure of the roots of *L. dichotomum* is very similar to that of the roots of *L. Selago*. The cells of the outer cortex have even thicker walls, and they stain slightly with cotton red, though much less deeply than does the xylem in the same sections.

Summary of Results.

The conclusions I have arrived at concerning the roots of *L. Selago* may be summed up as follows:—

1. The roots arise near the apex of the stem, but below the first leaves, before the vascular elements of the stem have become differentiated.

2. The roots are derived from a group of cells of the stem, the dermatogen of the root from several cells of the innermost layer of the stem periblem, the periblem and plerome of the root from the plerome of the stem.

3. The apex of the root is divided into three meristematic regions. The dermatogen divides to form both the root-cap of several layers and the epidermis; whilst the periblem forms a covering of about four layers of cells over the central plerome of the root.

4. All the cells of each layer are capable of division, and growth by apical cells does not take place.

5. The roots arising at the apex of each upright stem pass at first

obliquely, then directly down through the middle cortex. They only appear at the outside beneath the soil and on the under side of the obliquely growing stem.

6. Normally the roots are arranged in the cortex on all sides of the stele, but where the stem has grown obliquely to the ground plane all the roots are found in one half of the cortex of the stem.

7. The roots of this species do not dichotomize before leaving the cortex of the stem.

8. The bulbil, while still on the stem, may show the origin of two roots. The first-formed root curves round in the cortex, and, after separation of the bulbil from the stem, occupies a central position at the base of the green shoot.

9. Each root is connected with two protoxylem groups of the stem and the enclosed phloem. Exceptionally one may be in connexion with three groups of protoxylem, but never with only one. Leaf-traces, on the contrary, are never connected with more than one set of protoxylem elements.

10. The roots may be diarch or tetrarch, the metaxylem being arranged in two parallel bands in the former case, and in the shape of a horse-shoe in the latter. The protoxylem may occur all round the outer edge of the metaxylem, leading to the production of types intermediate between tetrarch and diarch. A diarch root may become tetrarch, or vice versa.

11. The protoxylem is formed of spiral and annular vessels; the metaxylem is of wide tracheids, either scalariform or with several rows of pits. The endodermis is of two or three layers, the cells of the innermost layer having thickened radial and transverse walls. The middle cortex shows large lacunae, which, however, do not communicate with those of the middle cortex of the stem. The roots are provided with a firm outer cortex of thick-walled cells, surrounded by the mucilaginous remains of the cells of the stem which have been decomposed by the root-cap in the downward growth of the root.

In conclusion, I wish to acknowledge my indebtedness to Prof. F. E. Weiss for his kindness in advising me with regard to this piece of work, and for the helpful criticism he has given.

EXPLANATION OF PLATE III.

Illustrating Miss Saxelby's paper on *Lycopodium Selago.*

pl. = plerome, *c.* = cortex, *i.* = intermediate layer (probably endodermis), *e.* = endodermis, *v.* = protoxylem, *ph.* = phloem, *x.* = xylem group, *st.* = stele, of stem, *r.pl.* = plerome, *r.pb.* = periblem, *r.d.* = dermatogen, *r.c.* = cap, *in.* = inner cortex, *m.* = middle cortex, *o.* = outer cortex, of root, *lt.* = leaf-trace, *c.c.* = concave, *c.v.* = convex, surface of bulbil.

Fig. 1. Transverse section of stem, showing centre of origin of very young root. × 85.

Fig. 2. Transverse section of stem, showing centre of origin of slightly older root; protoxylem of stem (*v.*) differentiated; base of leaf-trace (*lt.*) also shown. × 85.

Fig. 3. Transverse section of stem with still older root origin; endodermis of stem with thickened inner and radial walls. × 85.

Fig. 4. Transverse section near apex of stem bearing sporangia, showing base of root connected with phloem of stem, but not yet with xylem groups; *m.* = mucilaginous remains of cells destroyed by root-cap. × 43.

Fig. 5. Vertical section of young shoot grown from bulbil, showing young root origin; dermatogen of root not yet divided to form root-cap. × 85.

Fig. 6. Vertical section through apex of older root, showing origin of root-cap from dermatogen; periblem two layers deep over apex. × 85.

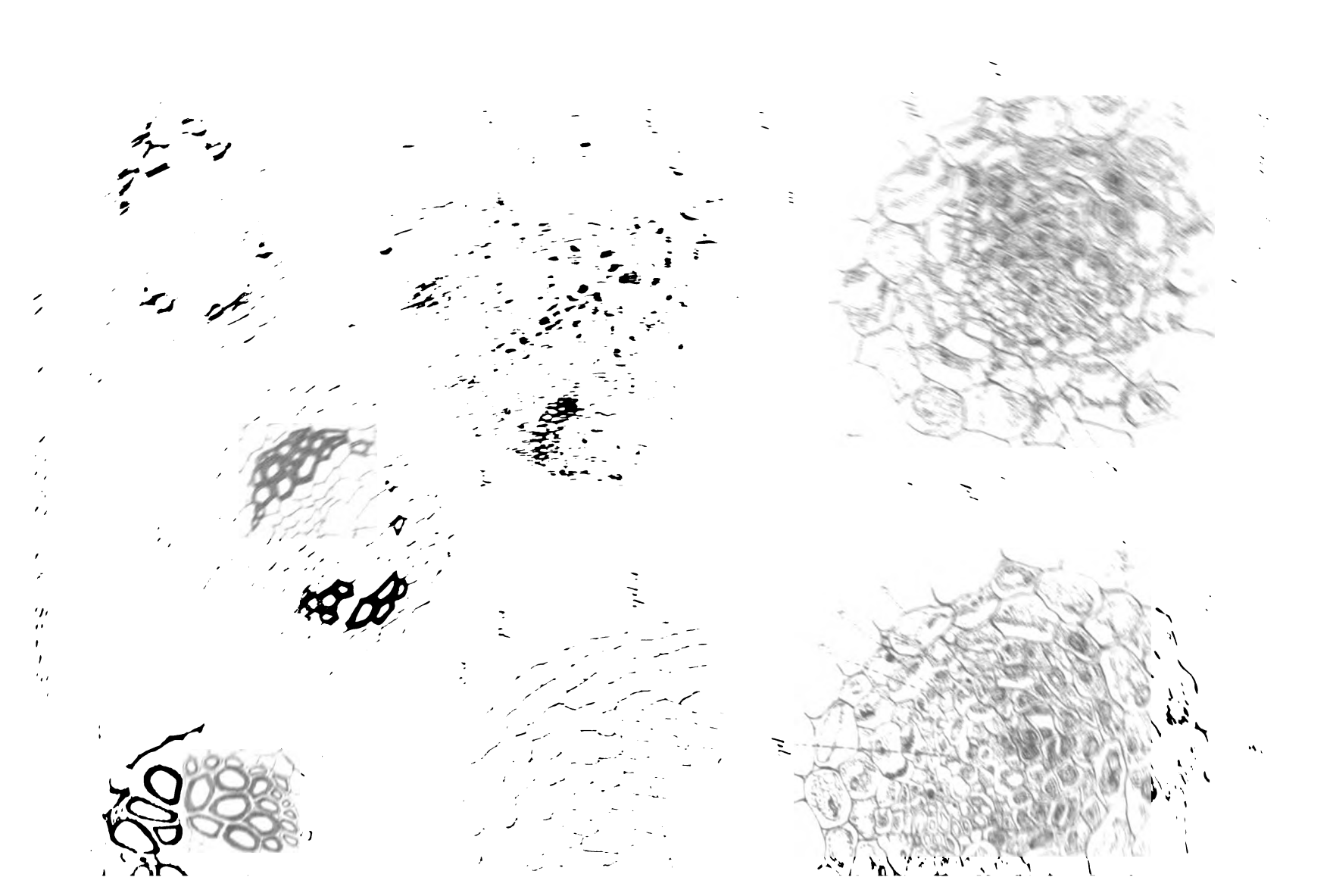
Fig. 7. Transverse section of obliquely growing stem, just above the level of the ground, showing roots all in one half of the stem cortex. This figure also shows the arrangement of the root-bundles with the convex side of the xylem towards the stem stele, the diarch type predominant. The section is cut from the part above the highest point at which a root appears outside the stem. × 22.

Fig. 8. Vertical section through bulbil and base of young shoot, from concave to convex side of bulbil, showing lateral origin of first root, and the same root cut transversely below the curve which has carried it into a terminal position. The section is cut in a direction almost parallel to the ground plane, though below the middle of the bulbil. The bulbil must therefore have been fixed on one side, and the root has described a double curve instead of growing straight down. × 22.

Fig. 8 *a*. Bulbil and shoot from which was cut the section figured in Fig. 8. Natural size.

Figs. 9, 10, 11. Transverse sections of vascular bundles of roots in cortex of older stem. Figs. 9, 10, young roots with metaxylem still unlignified. Fig. 9, tetrarch root with additional protoxylem elements outside the metaxylem. Fig. 10, diarch root. Fig. 11, older root of triarch or intermediate type. × 369.

Fig. 12. Transverse section of old root from cortex of stem, to show three cortical regions of root. × 85.



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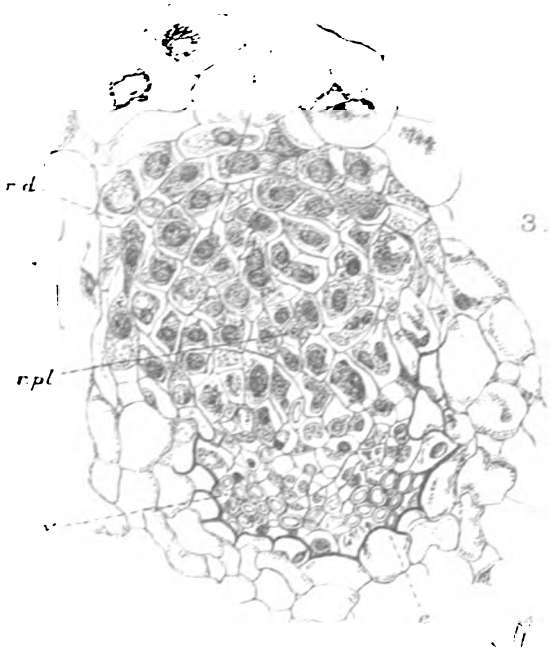

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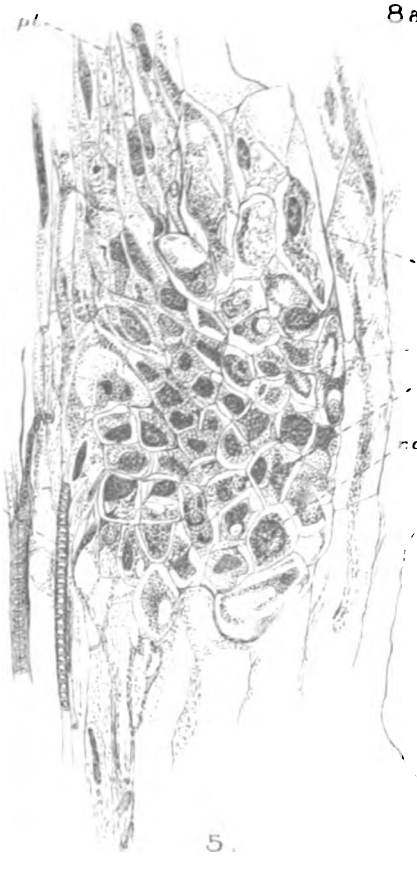

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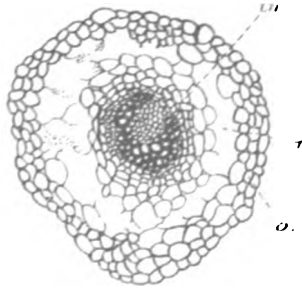

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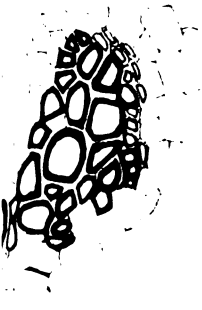

7.

Contributions to the Cytology of Humaria rutilans, Fries.[1]

BY

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With Plates IV and V.

HUMARIA rutilans,[2] Fries (*Peziza rutilans*, Fries) is an orange Discomycete 0·5–1 cm. in diameter, occurring in abundance on sandy soil among moss.

Material was collected during the autumn and winter of 1905 and 1906, and was fixed in the field, chiefly in Flemming's weaker fluid. It was embedded either through chloroform or through cedar oil, and was allowed to remain in the bath for from half an hour to two days at a temperature of about 54° C. Material did not appear to be adversely affected by the longer time, but it cut readily after half an hour, and this period was employed during the latter part of the work.

Sections were cut from 4 to 20 μ in thickness, and were stained either with Flemming's triple stain or with Heidenhain's iron Haematoxylin and a solution of erythrosin in clove oil.

I have to thank Miss H. S. Chambers for valuable help in the preparation of material during part of the work.

Development of the Ascocarp.

The young ascocarp is first distinguishable as a small knot of septate hyphae; sometimes one cell is larger than the others (Pl. IV, Fig. 1), but the nuclei are quite similar, and any distinction in size is soon lost. The outer cells are distinguished by their rather thick walls, the inner by their richer protoplasmic contents (Fig. 2).

Great difficulty was experienced in obtaining the very young stages; specimens, even, in which the asci had begun to form can hardly be dis-

[1] Thesis approved for the Degree of Doctor of Science in the University of London.

[2] In naming this fungus I have followed the diagnosis given by Rabenhorst's Kryptogamenflora; my specimens probably belong to the variety *vivida* of Nylsen. The species appears to be the same as that investigated by Guillermond, who, however, does not give the authority. I wish to thank Miss A. Lorrain Smith for her kindness in confirming my identification.

tinguished, under a lens, from the white or yellow sand grains among which they grow, and the youngest stages were only secured when they remained attached to an older ascocarp after the sand had been cleaned away.

The hypothecium is formed as a loose tangle of septate hyphae, which show somewhat scanty cytoplasm and a few granules. Each cell contains one or a few nuclei. These show a reticulum with conspicuous net-knots; a nucleolus may or may not be present; evidence of the occurrence of a central-body, as described by Harper (31) for *Phyllactinia*, or of the attachment of the chromatin filaments at a particular point, was not obtained.

Nuclear divisions in the hypothecium are karyokinetic (Fig. 3); the chromosomes are densely massed on the spindle and could not be counted; early prophases were not identified with certainty.

The nuclei at this time are of two sizes, and the smaller nuclei are seen to *fuse in pairs*, thus giving rise to the larger. Such fusions are very readily observed in all but the youngest ascocarps, and show the usual series of dumb-bell shaped figures (Figs. 4, 5). Migration of a nucleus from one cell to another occasionally takes place (Fig. 6), no doubt in connexion with these fusions; but, whereas more than twenty fusions were counted, migration was only twice observed. It seems likely, therefore, that it is not of general occurrence, and that the two nuclei which fuse have often been present in the same cell since their formation.

Ascus Formation.

The paraphyses are now differentiated, and, soon after, the first asci appear. In the subhymenial layer nuclei of two sizes are present, the larger being in the ascogenous hyphae, the smaller in the paraphyses and the cells from which they arise. The nuclei of the ascogenous hyphae at first resemble the fusion nuclei of the hypothecium in size and general structure (Fig. 7 *a*), and there seems no reason to doubt that they correspond to them, and that the nuclei in the paraphyses are to be related to the nuclei which have not undergone fusion.

As the ascogenous hyphae develop, their nuclei increase in size, and become vacuolate in structure (Fig. 7 *b*). Eventually the two terminal nuclei of the hyphae undergo simultaneous karyokinetic division. This may take place, either before (Fig. 10), or after (Figs. 8, 11), the hypha bends over to form the crozier first described by Dangeard (11) in 1894. In the early prophases of this division the nucleus shows a definite spireme, and large, centrosome-like bodies are present (Fig. 8). The spireme breaks up into about sixteen curved chromosomes (Fig. 9). These become densely massed on the spindle (Fig. 10), and eventually pass to the poles (Fig. 11). A terminal, uninucleate and a penultimate, binucleate, cell are formed, the two nuclei in the latter being sisters respectively of those in the terminal cell and the stalk-cell.

The ascus-cell, or cell from which the spore-containing part of the ascus grows out, is semicircular in section. A small projection, rich in cytoplasm, is formed from it, and grows actively, pushing up among the paraphyses and the bases of the older asci. Often the convex surface of the ascus-cell faces to one side or directly towards the hypothecium, and the projection may become much curved. Finally it assumes the characteristic shape of the ascus, and either before or after fusion the nuclei of the ascus cell pass into it.

Frequently the terminal cell cut off from the ascogenous hypha continues its growth (Fig. 12), giving rise, in the usual way, to another ascus, the terminal cell connected with which may in turn develop further (Fig. 13). It at first appeared that, in such cases, the nuclei of the new ascus were necessarily of the relationship of cousins. But, since the publication of my preliminary note on *H. rutilans* (20), another process has been observed. The growing terminal cell often lies in contact with the stalk-cell of the same ascus, and may become united to it by an H-connexion (Fig. 14). The nucleus of one of these cells may then migrate into the other (Fig. 15). If this takes place, the relationship of the nuclei of the new ascus is not necessarily close. It was not possible to ascertain whether such migration is of general occurrence. It was only observed once, and, in that case, the nucleus was passing from the terminal cell into the stalk-cell (Fig. 15); but, where proliferation has taken place, an H-piece was often found, and it was usually impossible to identify a nucleus in the stalk-cell of the corresponding ascus. Sometimes it is from the stalk-cell that the new hypha arises (Fig. 14).

The Meiotic Phase.

Very soon after the young ascus-cell has been cut off, its two nuclei enter independently upon the prophases of the first division. The stainable material of the nucleus forms a fine thread-work and becomes aggregated towards one side of the nuclear membrane forming the first contraction figure (Fig. 16), as has been described for the spore mother-cells of higher plants.

As this contraction passes off the thread thickens, and becomes more or less equally distributed (Fig. 17). A certain degree of polarity is sometimes observed (Fig. 18), the emptier part of the nucleus being remote from the region of the previous contraction.

As the thread distributes itself over the nuclear cavity, indications of a longitudinal split are here and there observed (Fig. 17). This becomes increasingly evident (Fig. 18), until the thread appears to be double along its whole length (Fig. 19).

Throughout these mitoses, chromomeres could not be distinguished, though the thread has frequently a granular appearance which no doubt indicates the usual arrangement of its constituents.

Before the longitudinal fission is complete, the two nuclei fuse, forming

the definitive nucleus of the ascus (Fig. 18). The nuclei lie against each other, either in the ascus or in the cell from which it originates, and appear simply to flow together; the two nucleoli are visible for a time, then they also fuse. The two spiremes mingle and cannot be distinguished after the first stages of fusion.

The synapsis or second contraction now sets in (Fig. 20). The chromatin filament thickens, the longitudinal split is more or less obliterated, and the whole thread, except a few loops which run out from the main mass to the periphery, becomes aggregated towards one side of the nucleus. The synaptic stage apparently persists for some time, then, as the contraction loosens, the loops running out from the central mass become more obvious, especially as shown in transverse section of the ascus (Fig. 21), and the longitudinal fission is once more apparent (Fig. 22).

The spireme next breaks up into its constituent loops, each of these forming a bivalent chromosome (Fig. 23), in the limbs of which the longitudinal fission can still be distinguished. The limbs may be twisted on each other, or united at both ends, forming a ring-shaped figure, or they may diverge very considerably (Fig. 24). Most of the forms described for the heterotype chromosomes of Phanerogams have been observed both at this and at later stages. All the chromosomes are not necessarily formed from loops, but their limbs are always derived from different portions of the spireme.

The chromosomes now shorten and thicken, and the longitudinal split is, for the most part, obliterated, though it may still be distinguished in favourable cases. During this process the chromosomes become arranged about the periphery of the nucleus, appearing to undergo a mutual repulsion. As the contraction of the chromosomes proceeds, it becomes possible to count them; they appear, as first stated by Guillermond (24) in 1904, to be sixteen in number (Fig. 25).

During synapsis the nucleolus becomes closely pressed against the nuclear membrane, and assumes a characteristic sickle shape (Fig. 22). When the synaptic contraction loosens it remains close to the wall, and either retains its irregular shape or becomes once more rounded. By the time the chromosomes are fully formed, it is seen to be vacuolate in structure, but it persists, though diminished in size, till the late telophases of the division (Fig. 33).

Spindle formation follows closely the process described by Harper (28) for *Erysiphe*, but the fibres are very delicate. The first stage to be recognized with certainty shows the two discoid centrosomes lying rather close together, with a separate cone of fibres radiating from each (Fig. 26). The centrosomes move apart, a spindle is established between them (Fig. 27); the radiating fibres become attached to the chromosomes, and have the appearance of drawing these on to the spindle (Fig. 28). A little

later the centrosomes reach opposite ends of the nucleus, and the monaster is formed.

Up to this time the membrane has been perfectly distinct and the space enclosed by it almost free from granules, but, from now onwards, the nuclear area becomes much less evident, though it can still be traced for some time.

The aster is demonstrated only with difficulty, but in favourable cases faint radiations may be seen passing away from the centrosome to be lost in the cytoplasmic reticulum.

The mature chromosomes, though so much contracted that the fact is more or less disguised, are typically V-shaped bodies, both limbs of which have undergone longitudinal fission throughout their length. Each chromosome lies on the spindle in such a way that one of its limbs is directed to either pole; the two limbs then separate, the bivalent chromosome being thus *transversely* divided. In the meantime, the longitudinal split becomes evident (Fig. 30), and each half of the bivalent chromosome thus passes to the pole as a V-shaped structure (Fig. 31). Sometimes one limb of the daughter chromosome remains attached to its fellow on the opposite half of the spindle after the other has broken away; the attached limb becomes considerably drawn out, and irregular figures are thus formed (Fig. 30 *a*).

The chromosomes become closely aggregated at the poles (Fig. 32), and reconstruction of the daughter nuclei begins. During this process the outline of the chromosomes can for some time be distinguished (Figs. 33, 34).

In the prophase of the second division, sixteen stout V-shaped chromosomes, no doubt representing those of the first telophase, reappear. They become attached to the spindle either at the apex of the V, or, more usually, about half way along its limbs (Fig. 35). The limbs separate and pass to opposite poles, being straight or bent according to the position in which they are attached to the spindle; the bent chromosomes are of much the more common occurrence (Fig. 36).

The longitudinal fission begun in the prophase of the first division, before the formation of the definitive nucleus, is thus completed.

Spindle formation takes place as in the first division, and the centrosomes, here also, are large and discoid.

The four nuclei of the ascus now pass into a stage of rest which, as it was frequently encountered, probably lasts for some time.

The Third Mitosis.

At the beginning of the third division the chromatin of each nucleus forms a delicate spireme (Fig. 37), which breaks up into sixteen curved chromosomes (Figs. 40, 41). Rounded bodies are present either near together (Fig. 38), or at opposite ends of the nucleus (Fig. 39); they

resemble the bodies observed during the divisions in the ascogenous hyphae; definite radiations could not be traced from them.

A spindle is formed and the V-shaped chromosomes become aggregated on it. The metaphases (Fig. 42) and anaphases (Fig. 43) of this division are difficult to obtain, and no doubt take place with rapidity; but the telophase was very frequently observed, and, in polar view, the number of chromosome ends radiating from the centrosome could be very readily determined. These ends were counted in some eleven cases, and were found to be always sixteen in number (Figs. 44, 45). During the anaphases and telophases the chromosomes are rather closely massed, but individuals were frequently traced throughout their length, and were always found to be curved, usually V-shaped structures (Fig. 43, 45). The sixteen ends counted in the telophase therefore represent *eight bent chromosomes*, their apices towards and their limbs radiating from the pole of the spindle.

An appearance in the telophase such as that shown in Fig. 44 might be due to the presence of sixteen rod-shaped bodies produced either by the transverse fission, or by the longitudinal fission, straightening and considerable contraction of the sixteen chromosomes of the prophase. No evidence in support of such a conclusion has been obtained, and the presence of V-shaped chromosomes in the later stages of division is against it. Guillermond (25) has recently stated that sixteen chromosomes pass to each pole in the third mitosis; but he neither figures the full number nor gives a detailed description of their appearance.

According to the present observations the sixteen chromosomes do not undergo fission in the metaphase, but half their number pass bodily to each pole of the spindle. The chromosomes of the prophase may either have arisen (1) by the breaking up of the spireme directly into sixteen parts, in which case the sixteen chromosomes would be presumably of different value, and the two daughter-nuclei would differ; or (2) by the breaking up of the spireme into eight parts, each of which, either before or after its separation from the others, undergoes longitudinal fission. The chromosomes would then form eight pairs of duplicates and the two daughter-nuclei would be equivalent. Occasionally the spireme shows two portions of the thread running parallel for a little distance (Fig. 37), but there is no evidence that this is due to a longitudinal split rather than to a chance looping of the thread. It thus appears that, in the third mitosis, sixteen diverse chromosomes are formed, and eight of these pass to each pole of the spindle.

SPORE FORMATION. Towards the end of the third mitosis the cytoplasm becomes rather densely massed at the poles of the spindles (Fig. 46) and shows an indication of faint lines radiating from the centrosome. The daughter-nuclei, as they separate, have the appearance of pushing actively into these masses, the cytoplasm seeming to flow back on each side of the nuclear beak (Fig. 47).

A little later the beak reaches the periphery of the mass and the outline of the latter is defined, in section, by a line passing in both directions away from the centrosome (Fig. 48). Frequently this line cannot be traced to the lower part of the spore, and this portion appears to be delimited by the sides of neighbouring vacuoles. The wall of the spore becomes increasingly definite and the nuclear beak continues to elongate, remaining attached to the inner membrane of the spore (Fig. 49). The nucleus then becomes rounded up and the beak disappears.

One or two vacuoles are always enclosed in the spore plasm, and become very conspicuous in the mature spore. They contain oil.

ABNORMALITIES. Certain abnormalities in the development of the ascus are fairly common ; trinucleate (Fig. 50) and tetranucleate (Fig. 51) asci are sometimes formed ; their fate could not be determined. In three or four cases asci were found containing two nuclei, each a good deal smaller than the ordinary definitive nucleus, and each undergoing synapsis (Fig. 52), and, in one case, a single nucleus of ordinary size was seen in which the contracted thread was aggregated into two separate masses (Fig. 53).

Occasionally after the second division, and more rarely after the first, two of the nuclei in the ascus undergo fusion (Fig. 54). It seems probable that such asci degenerate ; they were never observed at a later stage of development.

Two or more nuclei are sometimes observed within one spore (Fig. 55), binucleate spores being of fairly common occurrence.

SEXUALITY OF THE ASCOMYCETES.

The most important of the earlier work on this subject is due to De Bary (14, 15), who described the sexual organs of *Sphaerotheca*, *Aspergillus* and *Erysiphe*. From these and similar discoveries he inferred that a sexual process was of general occurrence among the Ascomycetes. In 1884, however, in view of his own and his pupils' more extended work, he (16) reached the conclusion that, while some of the Ascomycetes are undoubtedly sexual, yet others are parthenogenetic, the archicarp being present but developing without ordinary fertilization, or apogamous, the sexual organs having entirely disappeared.

These conclusions have been largely borne out by subsequent discoveries.

Harper (27), in 1895, observed the fusion of the sexual nuclei in *Sphaerotheca* and in 1896 in *Erysiphe*. The former discovery, contradicted by Dangeard (12) in 1897, was subsequently confirmed (Blackman and Fraser (6)) in 1905.

In 1900 Harper (30) described the coenocytic ascogonium and antheridium of *Pyronema* and the fusion of the numerous sexual nuclei in pairs.

In 1905 Claussen (10) announced fertilization in *Boudiera*, and in the same year Harper (31) gave a very full account of the sexuality and general cytology of *Phyllactinia*.

Besides these forms, in which the fusion of the sexual pronuclei was actually observed, several cases have been described among the lichens, the Laboulbeniaceae and the Ascomycetes generally, in which considerable evidence of such a process has been obtained.

In 1906 the behaviour of the nuclei in a 'parthenogenetic' form, *Humaria granulata*, was observed (Blackman and Fraser (8)). An ascogonium but no antheridium is developed, and the female nuclei fuse in pairs before passing into the ascogenous hyphae.

In 1907 a similar process was described by Welsford (4) for *Ascobolus furfuraceus*, and in the same year *Lachnea stercorea* (Fraser (21)) was added to the 'parthenogenetic' or homoiogamous forms.[1] In this case, however, reduction has progressed rather less far than in the others, since an antheridium is still present, though its nuclei degenerate *in situ*.

In all these forms, whether normal or reduced, the ascogenous hyphae arise from the ascogonium only, and the nuclei which pass into them are formed by the fusion of postmeiotic nuclei in pairs. They constitute the sporophyte generation and develop parasitically at the expense of the gametophyte.

The ascocarp of *Humaria rutilans* reaches maturity without the formation of sexual organs. In the hypothecium fusion of nuclei in pairs takes place. Ascogenous hyphae are then developed altogether similar to those which form the sporophyte in species with normal sexuality and moreover containing, as will be shown later, nuclei with the premeiotic number of chromosomes.

Thus it appears that here also the ascogenous hyphae form the sporophyte and that the fusions in the hypothecium constitute a reduced sexual process comparable to that observed by Farmer and Digby (17) in the prothalli of *Lastrea pseudomas* vars. *polydactyla*. In *Lastrea* the cells are uninucleate and the nuclear fusion is preceded by migration; this also is sometimes the case in *Humaria*, though, since the cells may be multinucleate, it perhaps does not always occur.

Humaria rutilans is thus an example of the so-called apogamous development of the ascocarp, or pseudogamy.

Probably a similar fusion of vegetative nuclei takes place in other Ascomycetes, such as *Cordyceps* and *Claviceps*, which have been described

[1] The various forms of reduced fertilization are grouped by Farmer and Digby (17) under the general term *pseudapogamy*. The diversity of such processes, among Fungi at any rate, having appeared to render desirable a further subdivision, the following terminology was recently (Fraser and Chambers (21 *a*)) suggested:—Fusion of two sexual nuclei of the same kind—*homoiogamy*. Fusion of one sexual and one vegetative nucleus—*hylogamy*. Fusion of two vegetative nuclei—*pseudogamy*.

as reaching maturity without the formation of sexual organs. The small size of the nuclei will in many cases, no doubt, render the actual observation of this process very difficult.

Formation of the Ascus.

De Bary (14, 16) first observed the presence of a single nucleus in the comparatively young ascus. He discovered that it underwent three successive divisions and that, about each of the eight resultant nuclei, a spore is normally organized. His observations were confirmed by Strasburger (39), by Schmitz and by Gjurasin (22), who also discovered in *Peziza vesiculosa* that the nuclear divisions are karyokinetic, and that well-marked asters are present.

In 1894 Dangeard, investigating *P. vesiculosa* and some other forms, made the important observation that the ascus originates from the binucleate, penultimate cell of an ascogenous hypha, and that the two nuclei of this cell fuse to form the definitive nucleus of the ascus. The bending over of the ascogenous hypha, the simultaneous karyokinetic division of the two terminal nuclei in such a way that the nuclei enclosed in the ascus shall not be of the relationship of sisters, and the fusion in the ascus have since been confirmed in a number of other species.

Recent research, however, has brought to light various modifications of this method. Maire (32), in 1903, discovered that, in *Galactinia succosa*, the two or three end cells of the ascogenous hypha are binucleate, the ascus being formed from the terminal cell; here also the two nuclei of the ascus are not sisters.

In *Peziza Catinus*, according to Guillermond (25), the bending over of the ascogenous hypha does not take place, otherwise development is typical.

Faull (19), in 1905, described, in *Genea hispidula* and a number of other species, the outgrowth of the ascus from the curved terminal cell of the ascogenous hypha; he regards this process as differing from the typical one only in the absence of a wall cutting off the recurved tip of the hypha. He makes no suggestion, however, as to the fate of the nucleus usually contained in the tip. He records the further interesting observation that in *Verpa bohemica* the ascus may grow out from the terminal cell of the hypha or it may arise from the second, third or fourth cell from the end, the growth of the hypha continuing beyond it. In one or two other forms the ascus may apparently spring from any cell whatever. Faull finds that the nuclei which fuse in the ascus, though not sisters, may be the daughters of sister nuclei.

Harper (31), both in *Erysiphe* and in *Phyllactinia*, found that the asci arise from binucleate cells of the ascogenous hyphae. There is no process of bending over, and no provision to prevent the inclusion of sister nuclei.

In *Humaria rutilans*, as in *Verpa bohemica*, the ascogenous hypha may

continue its growth, and it has been ascertained to give rise to other asci. I am inclined to think that the formation of the ascus from the subterminal cell of the ascogenous hypha is nothing more than a provision to allow the further growth of the latter, the asci developing, as is well shown in Fig. 13, and in Faull's Fig. 75, as lateral branches of the hypha. Probably the conjugate division of the two terminal nuclei of the ascogenous hypha, is a convenient method of providing two nuclei in the ascus and one in the terminal cell which is to continue its growth, rather than an arrangement to prevent the near relationship of the ascus nuclei. Where a cell contains more than one nucleus, simultaneous division seems to be the rule; it occurs, for instance, in the oogonium of the Oomycetes, in the embryo-sac of Phanerogams, and in the ascus itself.

The development of the ascus has been shown to be liable to considerable variation, the only constant process being the inclusion and subsequent fusion of two nuclei; even this is subject to exception, since in *H. rutilans* young trinucleate and tetranucleate asci are found.

The fusion in the ascus is regarded by Dangeard (12, 13) as sexual, and he has consistently denied the occurrence of a fertilization at any other stage in the life history of the Ascomycetes.

Recent research, however, has proved that, in a number of cases, a typical or reduced fertilization precedes the formation of the ascogenous hyphae. This in itself, unless the occurrence of two fertilizations in a single life history be regarded as possible, serves to disprove the sexual nature of the fusions in the ascus. In *Humaria rutilans* the fact that the reducing division begins before this fusion takes place, constitutes further evidence in the same direction.

For Harper (31) the fusion in the ascus serves to adjust the nucleo-cytoplasmic equilibrium. This indeed would account for the presence of more than one nucleus, since the ascus is to become the largest cell of a typically multinucleate mycelium, but it does not appear to explain their fusion.

In *Humaria rutilans* the nuclei lie in contact, and have entered on the prophases of division before fusion occurs. It may be suggested that, at this stage, the nuclear membrane becomes increasingly delicate, and the nuclei simply flow into each other, as is the case when nuclei artificially separated from their cytoplasm are brought into contact.

The Meiotic Phase.

Considerable additions to our knowledge of chromosome reduction among animals and higher plants have been made during recent years. In 1905, Allen (1) investigated the reducing division in *Lilium canadense*; he described the approximation and fusion in pairs of chromatin strands in the early prophase of the heterotype division. Synapsis then takes place, and is followed by a uniform distribution of the spireme.

A longitudinal split in the filament becomes evident, and was regarded by Allen as a separation of the threads which had previously fused. The spireme breaks up into a number of chromosomes which shorten and thicken while, at the same time, a second longitudinal split is distinguished. The limbs of the heterotype chromosome thus originate by a longitudinal split representing the separation of two fused, but originally independent filaments. The limbs of the chromosomes separate in the heterotype metaphase, and the second longitudinal split is completed in the homotype.

A similar account is given by Grégoire (23) and by Berghs (3) for various other Angiosperms, and by Allen (2) for *Coleochaete.*

In 1905 Farmer and Moore (18) published a full account of the reducing divisions or meiotic phase as studied by them in a number of animals and plants.

They observed stages corresponding to those seen by Allen, Grégoire and Berghs, but they were also able to distinguish various others, and have interpreted them quite differently.

According to these investigators, the heterotype division is initiated by the aggregation of the newly formed spireme towards one side of the nuclear area, forming the first contraction figure. As this loosens, the spireme undergoes longitudinal fission, but the split becomes more or less obliterated when the thread shortens and thickens in the synaptic or second contraction. During this process the nucleolus becomes vacuolate, and is regarded as giving up its substance to the chromatin element of the spireme.

Loops extend from the contracted mass to the nuclear wall, and in these the longitudinal split is still obvious. The sides of the loops become drawn into parallel positions; they are often twisted, and simulate the appearance of a single longitudinally split thread. This appearance is illusionary, since the earlier stages have been fully traced, and moreover the true longitudinal fission is still seen in the parallel sides. The synaptic tangle loosens, and the thread breaks up into chromosomes; each of these represents a loop or similar segment of the spireme, and the two limbs of each bivalent chromosome are thus derived from diverse portions of the thread which have been bent towards one another. The original longitudinal fission is still visible, but disappears as the chromosomes shorten and thicken. During this process the chromosomes pass to the periphery, appearing to act under the influence of mutual repulsion.

On the spindle the chromosomes break apart at their angle, the daughter chromosomes thus representing different portions of the spireme. In them the longitudinal split becomes once more apparent, and it takes effect on the spindle of the homotype division.

The meiotic phase is thus regarded as a peculiar series of events resulting in the reduction of the chromosome number and interpolated

between the longitudinal fission, characteristic of somatic divisions, in the first prophase, and the final separation of the split halves in the second metaphase.

The description of Farmer and Moore is in agreement with that given by Schaffner (38), and has been recently confirmed by Mottier (34).

The chief points in which these authors differ from Allen and Berghs are, (1) the occurrence of two contraction figures, (2) the approximation of the arms of each of the loops formed during synapsis, (3) the breaking up of the spireme so that each loop (or some equivalent portion of the thread) forms a bivalent chromosome, (4) the consequent transverse fission of each chromosome on the spindle. To a great extent the difference in interpretation is due to a difference in the seriation of the stages observed.

It has been suggested by various authors that the divisions in the ascus probably correspond to those in the spore mother-cells of higher plants, and also that the occurrence of three divisions is in some way related to the two nuclear fusions in the life-history of Ascomycetes.

In 1905, Harper (31), in his account of the development of *Phyllactinia*, described the two ascus nuclei, at the time of fusion, as each containing eight or nine chromatin threads attached to a central body. The centres fuse and the chromatin systems become intimately mingled. Synapsis takes place, the chromatin becoming contracted towards the central body. Later a spireme of eight strands is formed, and gives rise to the eight chromosomes of the equatorial plate. The chromosomes divide, and eight go to each pole. Eight chromosomes appear in the second and third mitoses, and in each case divide so that eight daughter chromosomes pass to the poles. These divisions appear to resemble the first, but are not initiated by a synapsis.

Maire (33), in 1905, described the first divisions in the ascus, in *Galactinia succosa*, *Pustularia vesiculosa*, and some other species, as respectively heterotype and homotype.

Guillermond (25), in the same year, investigated the ascus divisions of various species, amongst which is *Humaria* (*Peziza*) *rutilans*. He describes the nucleus of the ascus as rich in chromatin and containing a large oval nucleolus. The spireme often shows paired filaments; it undergoes a characteristic synapsis in which the chromatin is condensed at one side of the nucleus. Subsequently sixteen chromosomes appear, having the forms of U's or V's, with short, thick branches; they become grouped in the centre of the nucleus, and an intra-nuclear spindle and centrosomes appear; the asters can only exceptionally be distinguished. In the metaphase the chromosomes split longitudinally, forming hollow 'lozenges'; the two halves separate, and the V-shaped daughter chromosomes pass to the poles. In the anaphase sixteen V- or hook-shaped chromosomes were counted at each end of the spindle. On reaching the poles they branch and become united end to end, and the daughter nuclei are reconstructed.

The second mitosis begins by the formation of sixteen chromosomes; these become grouped on the spindle but do not acquire the lozenge shape of the first division. V-shaped figures only are seen, and later much drawn out filaments; there seems reason to believe that the V breaks at its apex as in the homotype division of Phanerogams.

The third division differs sensibly from the other two. A fine spireme is formed and divides into sixteen curved chromosomes, which become grouped in an equatorial plate. Guillermond states that, owing to the delicacy and large number of the chromosomes, the method of splitting could not be observed. In the late metaphase and early anaphase the chromosomes are elongated along the threads of the spindle, and directed towards the poles. In this division, as in the first and second, the number of chromosomes at each pole is given as sixteen.

I have been able, in the present investigation, to confirm Guillermond's observations with regard to the first and second mitoses. In the early prophases of the first division, and between the synaptic contraction and the appearance of the mature chromosomes, as described by him, I have been able to observe some further stages, thus bringing these divisions into line with the meiotic processes as described by Farmer and Moore. The first contraction and the longitudinal fission of the spireme (in part at least) take place before the fusion in the ascus, and the changes thus begun continue, apparently without interruption, in the definitive nucleus. The difficult question of seriation is here specially clear; it seems obvious, for instance, that such a stage as Fig. 17, where the ascus nuclei each show a few paired threads, must precede that shown in Fig. 19, where the spireme of the definitive nucleus is double throughout its length. Synapsis sets in, loops are formed, their sides approximate, and the whole loop constitutes a bivalent chromosome. On the spindle of the first division the two limbs of the chromosome break apart, thus separating unlike portions of the spireme. In the second division the longitudinal split takes effect.

Thus here, as in *Lilium candidum* and the other forms described by Farmer and Moore, the first meiotic division is diaschistic, and brings about a reduction in the sense of Weismann.

The Third Mitosis.

The processes in the ascus are confused, as compared to the meiotic phase in other organisms, by the introduction of a nuclear fusion.

The number of chromosomes in the vegetative divisions directly preceeding meiosis has been constantly found to be the same as in the first division in the ascus. This was ascertained by Harper for *Pyronema* (30) and *Phyllactinia* (31), both forms with normal sexuality, and in the present instance for *Humaria rutilans*.

In *Humaria rutilans*, at least, the heterotype division is begun before

fusion takes place, and, since no evidence of the union of the two spiremes was obtained, it may be supposed that each continues its development separately, and separately breaks up into the reduced number of chromosomes. There are sixteen chromosomes in the divisions in the ascogenous hyphae, and the sixteen which appear in the heterotype prophase are thus made up of two sets of bivalent chromosomes, eight of which have been derived from each spireme; in the same way the definitive nucleus of the ascus is a double structure, representing two nuclei enclosed within one membrane. This view is further borne out by the occasional appearance of a nucleus in which the spireme, in the second contraction, is aggregated into two masses, or of an ascus containing two nuclei in synapsis, which have no doubt failed to fuse, but have nevertheless continued their development independently.

The third mitosis has been regarded by Maire and Guillermond as vegetative, and Guillermond states that in *H. rutilans* the number of chromosomes in the third telophase, as in the first and second, is sixteen.

If, however, the conclusions drawn from the present research be correct, the sixteen ends radiating from the pole at this stage represent, not sixteen rods, but *eight* V-shaped chromosomes. The postmeiotic number, that is to say, has become apparent, and the fusion in the ascus is compensated.

Nemeč (35), experimenting with the root-apices of Phanerogams, found that treatment with 0·75 per cent. of chloral hydrate produced degeneration of the spindle fibres; cell division is thus inhibited, but the daughter nuclei separate and binucleate cells appear; in these the two nuclei either fuse, subsequent divisions showing double the somatic number of chromosomes, or divide simultaneously; in the latter case three cells are formed, the middle one containing two nuclei; these may fuse and show the double number of chromosomes in their divisions.

After a few hours mitoses showing double the somatic number of chromosomes cannot be identified, reduction having apparently taken place. Nemeč observed in *Pisum* a large cell, quite like those which contain two nuclei or a nucleus with the double number of chromosomes, containing a nucleus in the late anaphase, which showed the ordinary somatic number. This he regards as a reducing division. It seems quite possible that such divisions correspond to the third division in the ascus. This division resembles an ordinary vegetative mitosis, but shows in the metaphase the double number of chromosomes, and in the anaphase the ordinary number (half that shown in the metaphase). The relation of the two stages would not, however, be obvious, except where, as in the ascus, their connexion could be recognized in some other way. The process, compensating as it does a vegetative or asexual fusion, is much less elaborate than the meiotic reduction.

Evidence of similar fusions and reductions in graft hybrids has

been adduced by Noll (36), and we may hope here to obtain some knowledge of the effect of this method of chromosome distribution in heredity.

In *Phyllactinia* the number of chromosomes, or chromatin strands radiating from the central body, is the same throughout the life history of the fungus. On the fusion of the sexual pronuclei these strands combine in pairs; thus the diplocytic nuclei contain not $2n$ chromosomes, but n bivalent chromosomes. A similar process takes place in the fusion in the ascus, and the eight chromatin strands of the definitive nucleus are thus regarded by Harper as tetravalent and as actually representing thirty-two somatic chromosomes. The meiotic divisions[1] then take place and four nuclei are formed; each contains eight bivalent chromosomes representing sixteen somatic chromosomes. In the third division the valency of each chromosome is again halved and eight somatic chromosomes appear.

The essential facts are thus the same in *Phyllactinia* and in *Humaria rutilans*. In each a sexual fusion takes place (normal in *Phyllactinia*, much reduced in *Humaria*), and the actual number of chromosomes is thus doubled. Owing to the association of the chromosomes in pairs, the apparent number remains unchanged in *Phyllactinia*; in *Humaria* sixteen chromosomes, the premeiotic number, can be counted in the ascogenous hyphae. In each case a fusion of two nuclei takes place in the ascus, resulting in the association, in *Phyllactinia*, of eight tetravalent chromosomes within one membrane, in *Humaria*, no doubt, of thirty-two univalent chromosomes. Meiosis occurs and four nuclei are formed, each of which, in the next prophase, shows, in *Phyllactinia* eight bivalent chromosomes, in *Humaria* sixteen univalent chromosomes, the reduced number for *two* nuclei. In the third division a further reduction occurs, compensating, no doubt, the fusion in the ascus, and in each of the two species eight univalent chromosomes are shown in the anaphase.

It may be suggested that, once a third division, compensating the fusion in the ascus, had been established, the latter would become a necessary process, and this may account for the extraordinary regularity of the appearance and fusion of the two nuclei, just as the occurrence of meiosis is held to render imperative some form of reduced fertilization if the normal sexual process be lost.

An interesting transition between the two arrangements detailed above perhaps occurs in *Pustularia vesiculosa* as described by Maire (33). He states that eight chromatin bodies appear in the prophase of each of the three divisions, but unite into four as they pass on to the spindle. In the first anaphase separation occurs on the spindle, so that eight chromatin

[1] Harper did not observe a first contraction of the chromatin in the heterotype prophase; the absence of this stage may probably be connected with the early pairing of the chromosomes.

bodies pass to each pole; in the second and third anaphases only four can be distinguished. It may perhaps be possible that these eight bodies are not, as suggested by Maire, protochromosomes, but rather true chromosomes, which become associated in pairs during a part of first and second mitoses, as is the case throughout these divisions in *Phyllactinia*. The four bodies which pass to the poles in the third division would be, as in both *Phyllactinia* and *Humaria*, the univalent, postmeiotic chromosomes.

It must be added that Guillermond (25) finds eight chromosomes throughout the ascus divisions in *P. vesiculosa*. Possibly, then, the grouping of the chromosomes in pairs does not always take place; such an hypothesis would account for the discordant results obtained by Maire and Guillermond. It seems not improbable, on the analogy of the present researches, that the eight structures counted by Guillermond in the third telophase represent only four chromosomes.

In *Galactinia succosa* Maire describes the association of the chromosomes as lasting rather longer than in *P. vesiculosa;* this species, therefore, shows a state of affairs in closer accordance with that obtaining in *Phyllactinia*.

In *Phyllactinia*, if Harper's suggestions and those set forth above be correct, it seems probable that the constituents of a given double chromosome will pass to different nuclei, and it may be that their association in pairs is a provision to ensure that end. A similar provision then is evident in *G. succosa* and *P. vesiculosa*, and it is likely that it exists in *Humaria rutilans*, although in this case it is not apparent. If this be so, a sorting of the chromosomes, analogous to that which occurs in the meiotic phase, would appear to take place in the third division in the ascus.

This division represents a type of reduction differing markedly from the meiotic phase, and to which the term brachymeiosis may be conveniently applied. It shows none of the characteristic features of meiotic reduction, it takes place in a single mitosis, and it is not preceded by a contraction of the nuclear material.

The absence of this contraction probably entails the most essential distinction between meiosis and brachymeiosis. Meiosis, while it brings about the separation of entire somatic chromosomes, yet, in its contraction, presumably allows a mingling of maternal and paternal chromatin. In brachymeiosis such a contraction does not appear, and the separation of entire chromosomes alone takes place. It is thus to be expected that the product of a brachymeiotic division should follow very exactly the law of Mendel allowing none of the minor variations which meiosis makes possible. On the other hand it is quite likely that in brachymeiosis the *entire* nuclei which united in asexual fusion separate from each other, and that no interchange of chromosomes takes place.

The existence of brachymeiosis suggests a new distinction between sexual and asexual fusions. The two fusions in the life-history of *Humaria rutilans* are very similar in character; both are fusions of apparently undifferentiated nuclei, and, in both, the nuclei which fuse have often been present in the same cell since their formation. Each fusion also results in a doubling of the number of chromosomes, and is compensated by a process of reduction.

The first fusion, however, occurs at the same stage in the life-history as a normal act of fertilization and initiates a similar development. The second fusion takes place after meiosis has begun. The syngamous fusion is related here, as in all other investigated organisms to a meiotic reduction. The asexual fusion in the ascus is followed by the simpler brachymeiotic process, and there is reason to believe that this method of reduction may compensate other asexual fusions also.

Association of Nuclei.

According to Maire (32, 33) the nuclei in the ascogenous hyphae of various Ascomycetes are associated in pairs, and constitute a synkaryon comparable to that observed by various authors in the Basidiomycetes.

I have not been able to observe such an arrangement in *H. rutilans*.

Harper (31) also has observed paired nuclei in *Phyllactinia*, and suggests that the fusion in the ascus is to be related to the fusion in the teleutospore and basidium, the association of nuclei in pairs having 'worked back' in the latter cases, to the stage of fertilization, and the occurrence of two fusions, as in the Ascomycetes, being therefore eliminated.

As, however, Blackman and others (5, 7, 9) have shown, the nuclear union in the teleutospore is a stage in the act of fertilization initiated in the aecidium (or at some equivalent point in the mycelium), and corresponds to the fusion of the sexual nuclei in other plants and animals, and therefore to the fusion which takes place typically in the female organ of Ascomycetes.

The fusion in the ascus follows the fusion of the sexual nuclei. It appears to be a peculiar process intercalated in the life-history of the Ascomycetes and is compensated by the third division in the ascus.

The ascus, however, resembles the teleutospore (or its outgrowth, the promycelium) and the basidium in being a spore mother-cell in which reducing divisions take place.

Spore-formation.

The details of spore-formation were first studied by Harper (26), who regards the spore as cut out by astral rays which fuse laterally to form a membrane.

This point, together with its bearing on the phylogeny of the Ascomycetes, has recently received full discussion from Faull (19), who describes

the spores as delimited by the differentiation of a finely granular protoplasm, and from Overton (37), who confirms the description of Harper.

Faull's account is of great interest, but it does not seem to satisfactorily explain either the persistence of the astral rays or the formation of the nuclear beak; the latter appears in *H. rutilans*, as in the forms described by Harper, before the spore is delimited.

The exact function of the radiations from the centrosome is, owing to their extreme tenuity, somewhat difficult to ascertain in *H. rutilans*.

Farmer and Moore (18) regard the spindle fibres rather as protoplasm modified by the forces at work in the cell than as actively growing entities. For them the spindle is a passive manifestation of the real operating agency.

In *H. rutilans*, the spore is delimited by the astral rays, but it would seem that these represent not cell organs of the nature of cilia, as suggested by Harper (31), but rather currents set up in the neighbourhood of the centrosome as it pushes into the dense cytoplasm near the pole. It is in accordance with such a point of view that the boundary of the spore should sometimes be partly defined by the walls of neighbouring vacuoles, or, as would seem to be the case in the abnormal ascus of Fig. 55, by the ordinary cytoplasm.

Summary.

1. The ascocarp of *Humaria rutilans* originates as a tangle of septate hyphae; sexual organs are not differentiated.

2. Fusions of nuclei in pairs occur in the hypothecium constituting a process of reduced fertilization or apogamy. The cells containing the fusion nuclei form ascogenous hyphae.

3. Divisions in these hyphae are karyokinetic, showing sixteen chromosomes.

4. The first and second divisions in the ascus are respectively heterotype and homotype. They show the stages observed by Farmer and Moore in the meiotic phase of certain animals and plants, and they bear the same interpretation.

5. During the first mitosis fusion of the two nuclei in the ascus occurs. At this time the spireme in each already shows evidence of longitudinal fission.

6. Sixteen chromosomes appear in the first two divisions in the ascus and in the prophase of the third. They are regarded as representing two sets of post-meiotic chromosomes united with one membrane.

7. In the telophase of the third division eight chromosomes only are seen at each pole. The two sets of post-meiotic chromosomes have thus separated, and the reduced number is apparent. To this type of reduction the name *brachymeiosis* is given.

8. The spores are outlined by radiations passing from the centrosome; near the base of the spore vacuoles may take part in the process.

July, 1907.

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EXPLANATION OF PLATES IV AND V.

Illustrating Dr. Fraser's paper on *Humaria rutilans*.

Fig. 1. Section through a very young ascocarp, lying against the wall of an older one. × 625.
Fig. 2. Section through a rather older ascocarp. × 625.
Fig. 3. Mitosis in hypothecium. × 1250.
Fig. 4. Apogamous fusion in the hypothecium. × 1250.
Fig. 5. The same, later stages. × 1250.
Fig. 6. Nuclear migration in the hypothecium; nuclei of two sizes are shown. × 1250.
Fig. 7. Part of the subhymenial layer, showing nuclei in young ascogenous hyphae at *a*, and in a hypha just before crozier formation at *b*. (From the same ascocarp as Fig. 4.) × 1250.
Fig. 8. Early prophase in the ascogenous hypha. × 1875.
Fig. 9. Prophase in ascogenous hypha, showing sixteen chromosomes. × 2808.
Fig. 10. Metaphase in ascogenous hypha. × 1875.
Fig. 11. Telophase in ascogenous hypha. × 1875.
Fig. 12. Proliferation of terminal cell of ascus. × 1250.
Fig. 13. An ascus (*a*), the terminal cell connected with which has continued its growth and given rise to another ascus (*b*); from the terminal cell of which a third ascus (*c*) has arisen. 1250.
Fig. 14. *H*, connexion between stalk and terminal cell. The nucleus of the latter has passed into the stalk-cell, from which a hypha is growing out. × 1250.
Fig. 15. Nucleus migrating from the terminal into the stalk-cell. × 1250.

FIRST DIVISION.

Fig. 16. Two nuclei in the ascus, each showing the first contraction-figure of the heterotype prophase. × 1875.
Fig. 17. Two nuclei in the ascus; the beginning of the longitudinal fission can be distinguished in the spireme of each. × 1875.
Fig. 18. Fusion in the ascus, the longitudinal fission is further advanced. × 1875.
Fig. 19. Definitive nucleus of ascus; longitudinal fission complete. × 1875.
Fig. 20. Synapsis. Longitudinal section of ascus. × 1875.

Fig. 21. Synapsis. Transverse section of ascus. The arms of each loop are closely approximated. The longitudinal fission can just be distinguished in places. × 1875.

Fig. 22. Ascus in longitudinal section. Nucleus passing out of synapsis. The longitudinal fission is once more obvious. × 1875.

Fig. 23. Spireme broken up into chromosomes, in the limbs of these the longitudinal fission can be seen. × 1875.

Fig. 24. Immature chromosomes. × 2808.

Fig. 25. Nucleus showing sixteen mature chromosomes. × 2808.

Fig. 26. Spindle formation; the two centrosomes lie close together with a cone of fibres radiating from each. × 1875.

Fig. 27. Later stage, spindle is established. × 1875.

Fig. 28. Passage of chromosomes on to spindle. × 1875.

Fig. 29. Chromosomes on spindle; centrosomes and asters visible. × 1875.

Fig. 30. Chromosomes on the spindle. × 2808.

Fig. 31. Passage of chromosomes to poles. × 1875.

Fig. 32. Chromosomes aggregated at the poles. × 1875.

Fig. 33. Reconstruction of daughter-nuclei. × 1875.

Fig. 34. Same; later stage. × 1875.

SECOND DIVISION.

Fig. 35. Passage of chromosomes on to spindle. × 1875.

Fig. 36. Telophase; sixteen bent chromosomes. × 2808.

THIRD DIVISION.

Fig. 37. Early prophase, showing delicate spireme. × 1875.

Fig. 38. Spireme stage; two centrosome-like bodies. × 1875.

Fig. 39. Later spireme; centrosome-like bodies. × 1875.

Fig. 40. Chromosome formation in four nuclei. × 1875.

Fig. 41. Nucleus showing sixteen curved chromosomes. × 2808.

Fig. 42. Metaphase of third division. × 1875.

Fig. 43. Passage of curved chromosomes to poles. × 1875.

Fig. 44. Telophase; lateral view, and polar view in which the sixteen ends of eight curved chromosomes can be counted. × 2808.

Fig. 45. Another polar view showing the eight chromosomes. × 2808.

Fig. 46. Telophase in lateral view. × 1875.

Fig. 47. Later stage, formation of nuclear beaks has begun. × 1875.

Fig. 48. Spore formation. × 1875.

Fig. 49. Young spore. × 1875.

Fig. 50. Trinucleate ascus. × 1250.

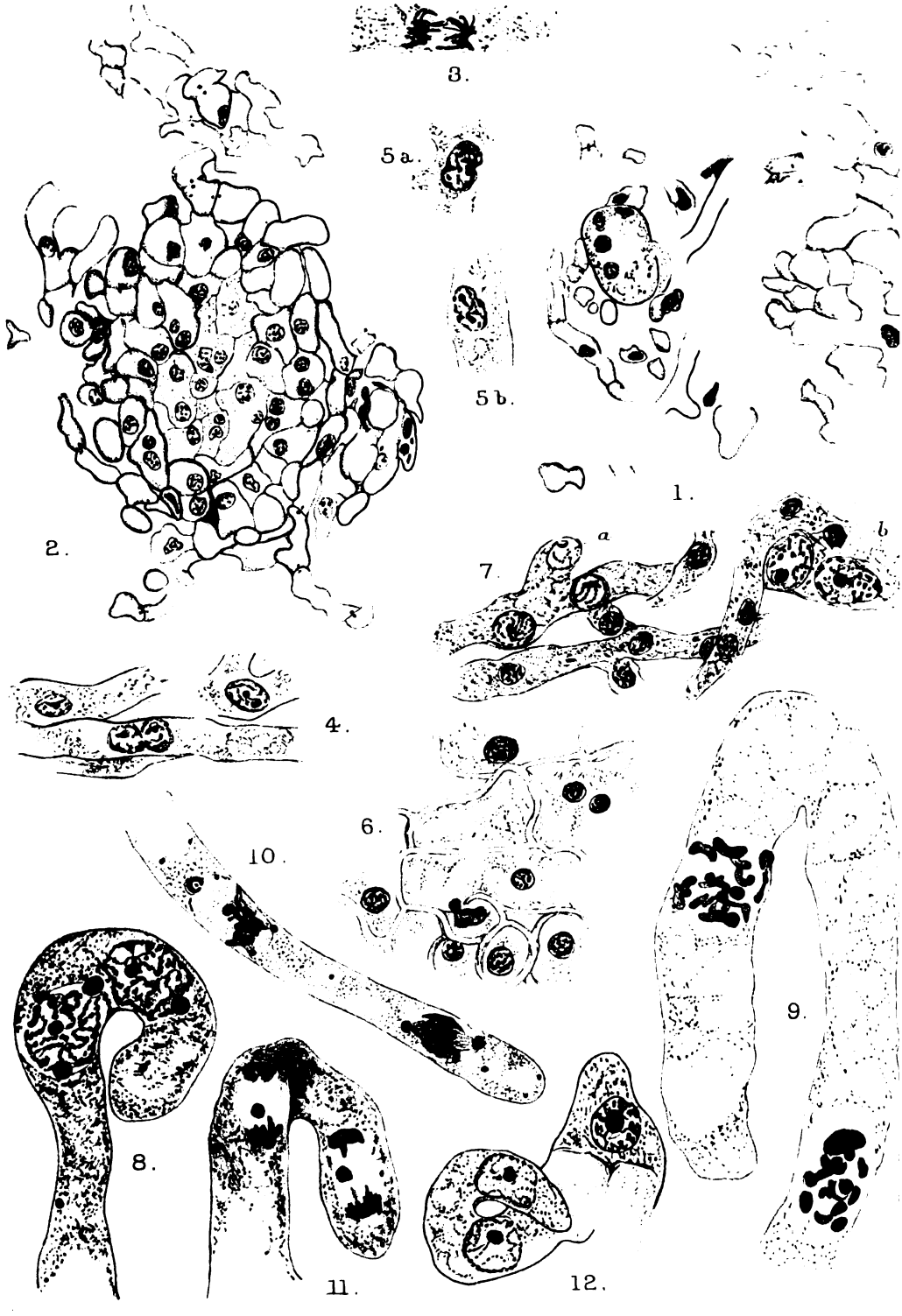
Fig. 51. Tetranucleate ascus. × 1250.

Fig. 52. Ascus containing two nuclei in synapsis. × 1875.

Fig. 53. Ascus containing a single nucleus in which the chromatin is aggregated into two masses. × 1875.

Fig. 54. Fusion between two nuclei in the ascus after the second division. × 1250.

Fig. 55. Ascus containing three normal spores and one with five nuclei. × 1250.



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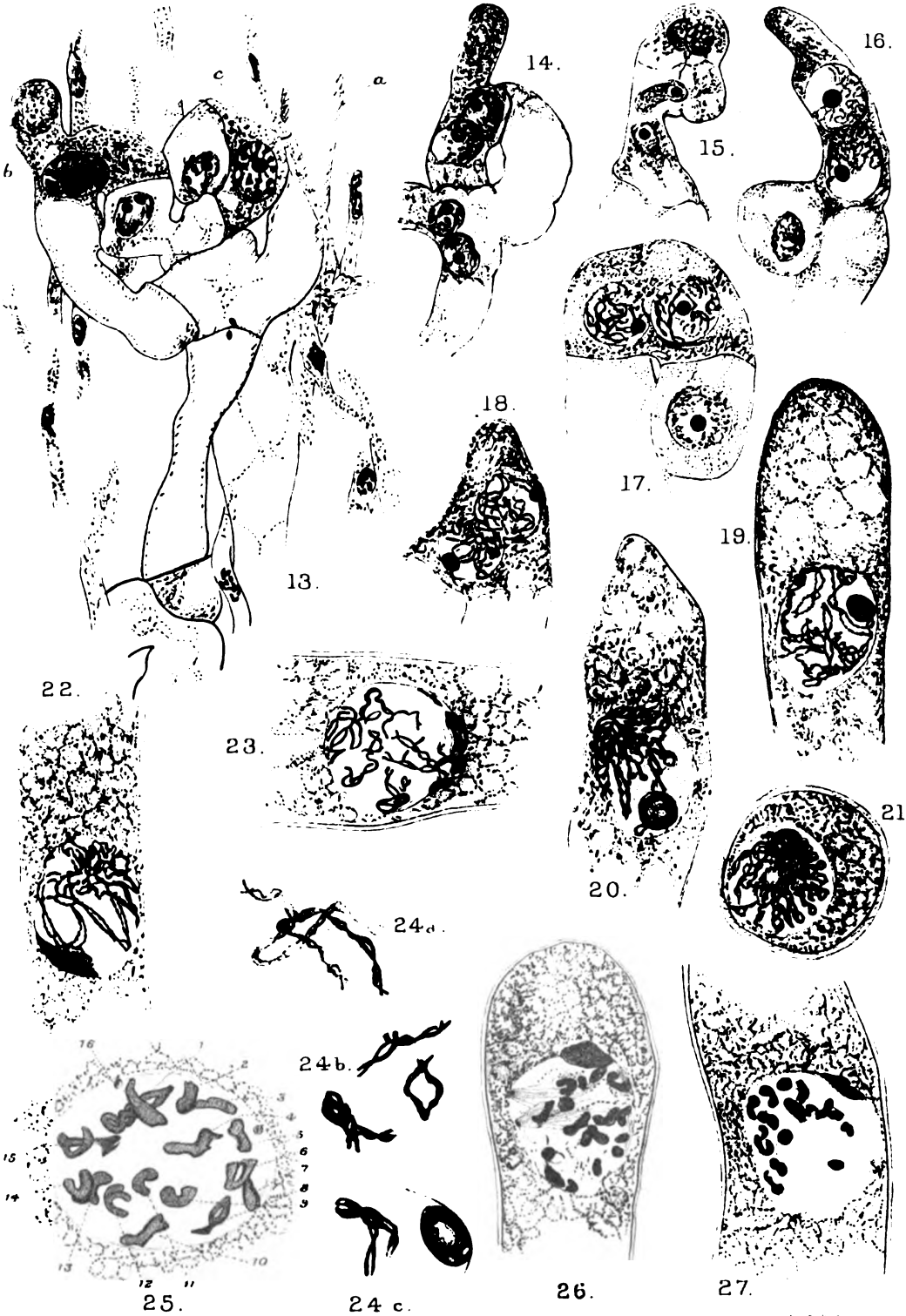
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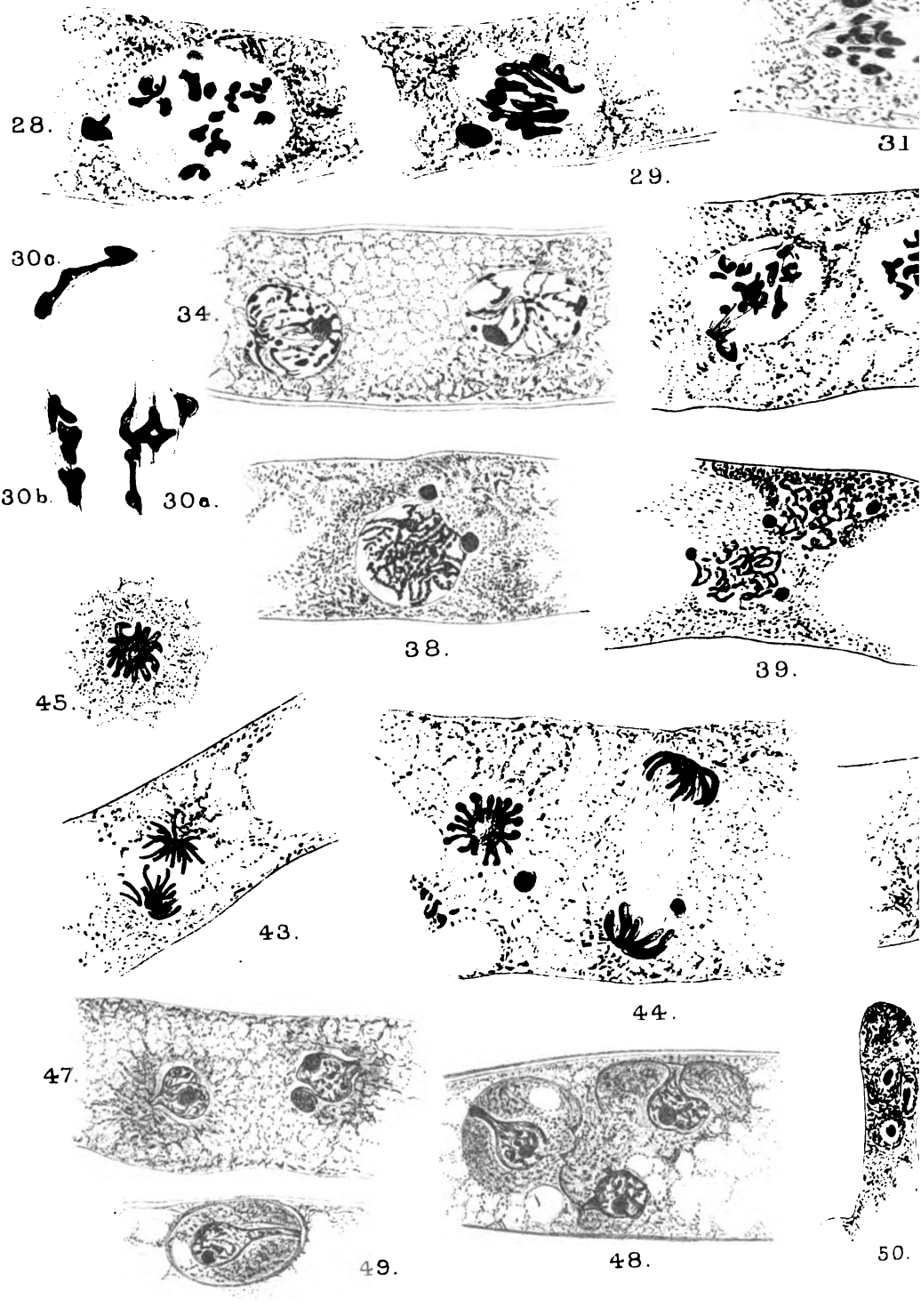

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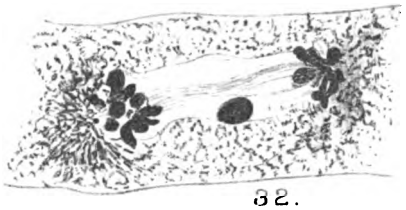

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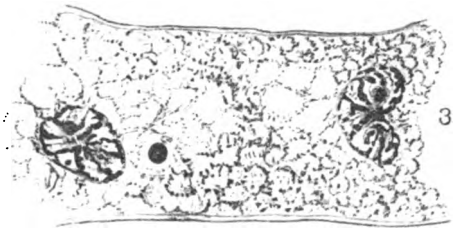

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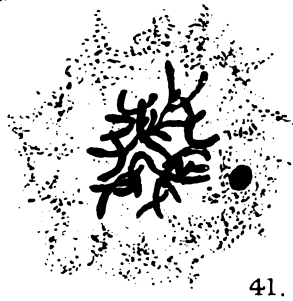

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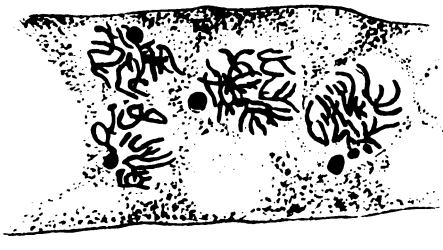

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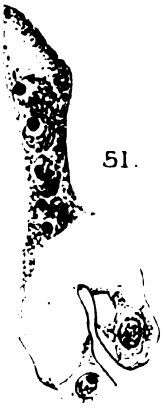

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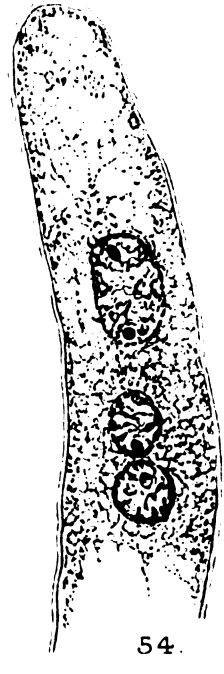

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On a New Pteridosperm possessing the Sphenopteris Type of Foliage.

BY

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With Plate VI.

THE discovery of the seed of *Lyginodendron* on the part of Professor Oliver and Dr. Scott,[1] followed more recently by that of the male fructification (*Crossotheca*) by Mr. Kidston [2], has rendered our knowledge of this fossil scarcely less complete than it would have been had the genus still survived at the present day. One or two points, however, remain to be determined. Of these perhaps the most important relates to the manner in which the seeds were borne. It will be remembered that, when the seed *Lagenostoma Lomaxi* was first attributed to *Lyginodendron*, no direct evidence of continuity could be demonstrated. But in the case of other Lagenostomas, subsequently described,[3] there did appear to be strong evidence in favour of the view that the seeds, like the male organs, were borne on fronds with reduced lamina.[4] The present specimen, though not obviously a member of the same genus, tends to confirm this conclusion in a somewhat emphatic manner.

The fossil frond figured here belongs to the series of Coal Measure fossils which constitute the Goldenberg collection, preserved in the Museum of Fossil Botany of the Swedish Academy of Sciences at Stockholm. I am indebted to my friend Prof. A. G. Nathorst, Keeper of the Museum, for facilities for examining this and other collections under his care, and I would here express my very sincere thanks to him for generously giving me permission to describe this specimen, and for permitting me to borrow it for that purpose.

Goldenberg's collection, part of which was illustrated in his 'Flora Saraepontana Fossilis,' published between 1855 and 1862, of which, however, only a portion ever appeared, was derived from the Saar-Rhein

[1] Oliver and Scott ('04). [2] Kidston ('06). [3] Arber ('05[1]). [4] Arber (05[2]).

Coalfield of Germany. The present specimen, collected many years ago, was not among those described by Goldenberg. The locality from which it was derived is given on the label as 'Judenschlag Graben'. The horizon is probably that of the Westphalian division of the Upper Carboniferous, using the term in the sense in which it is applied on the Continent. The fossil is labelled, probably by Goldenberg, as a new species of *Hymenophyllum*, and, in another handwriting, as a fertile *Sphenopteris*.

The specimen consists of a piece of shale, of uneven surface, about 20 cm. in length, showing on one side part of a fertile Sphenopterid frond with several seeds still in continuity. The more interesting portion of this frond is figured on Pl. VI, Fig. 1, magnified three times. On the reverse side, a leaf of an *Alethopteris*, no doubt *A. lonchitica* (Schl.), is seen. The anatomical structure is not preserved, the fossil being simply an impression. Although fragmentary in places owing to the uneven bedding of the shale, the preservation of the frond as a whole is quite satisfactory.

The portion of the leaf figured (Pl. VI, Fig. 1) is no doubt only a small part of a highly compound frond, just as is the case with the majority of impressions of the *Sphenopteris* type. A fragment of the rachis of the second, or some higher order, is seen on the right of the figure. Most of the pinnae are obviously detached, but in two cases, one of which is shown in the drawing, they appear to be still in continuity with the axis.

The frond as a whole appears to have been of a delicate, graceful type, the leaflets, like those of several other species of the same genus, being quite small and very narrow.

The rachis has a breadth of about 3 mm. The surface is faintly striated longitudinally, but shows no signs of the presence of any glandular organs, similar to those which often occur so abundantly on the rachis of *Sphenopteris* (*Lyginodendron*) *Hoeninghausi*, Brong. Another rachis of greater breadth is also associated, but it is by no means obvious that it had any real connexion with the frond under discussion.

The pinnae of the highest order, of which three are seen on Pl. VI, Fig. 1, were slender axes, which probably, in the living state, exceeded 5 cm. in length. None of the fragments of pinnae shown on this specimen are however complete. The pinnules were numerous, subopposite, and reached a length of 1 cm. or more. They were deeply divided into a number of very narrow, linear segments, of about 1·5 mm. in length, the lamina as a whole being very small, and probably considerably reduced in comparison with the, as yet unknown, sterile frond (Plate VI, Figs. 1, 3, 4, 5, 7, and 8).

The great majority of the pinnules seen in this specimen appear to have been fertile, the seeds being borne at the extremities of the linear segments or lobes (Plate VI, Figs. 4, 5, 7, and 8). The absence of seeds, in certain cases, is probably due to some accident during preservation.

Usually each of the segments bears a single seed, though more rarely they appear to have bifurcated, each lobe terminating in a seed.

The Seeds.

The seeds themselves are extraordinarily small objects. Two examples are figured on Plate VI, Figs. 2 and 3, magnified twenty times. Their average length is about a millimetre, the largest being 1·2 mm., while at their greatest width they measure ·75 mm. across. They seem to be of the radially symmetrical type. They are more or less oval in shape, and exhibit several rather sharp, longitudinal ridges (Pl. VI, Fig. 3). They do not appear to show any special apical peculiarities.

There would seem to be some grounds for the belief that most of the seeds seen on this specimen are enveloped in closely-fitting cupules (Plate VI, Figs. 4–8). It is, however, very difficult to decide whether such an organ is really present, for not only are the seeds very minute, but the cupules, if they exist, are closely similar to them in size and shape. In such impressions as these, it must always be difficult to demonstrate the presence of a cupule, unless it has, as in the case of *Lagenostoma Sinclairi*, Kidst. MS.[1], a form quite different from that of the seed itself, or unless it is a deeply cleft structure, as for instance in *Calymmatotheca Stangeri*, Stur [2]. In the present case, if cupules are present, they appear to correspond more closely to those of *Lagenostoma Lomaxi* [3], though they are perhaps less deeply divided, and without the glandular structures found on the cupule of that seed. Impressions of such cupules must naturally be difficult to distinguish from those of the seeds themselves.

In this specimen, there do not appear to be any good cases in which empty cupules, without seeds, can be recognized. On Pl. VI, Fig. 2, there is a somewhat indefinite body, seen to the right of the naked seed, which may be of this nature, but it is impossible to determine its precise nature.

In many instances, however (Pl. VI, Figs. 5–8), there are indications which suggest the presence of an outer investment containing a seed, the surface of which does not appear to correspond exactly with that of the naked seed. In such cases, the shape of the supposed cupule differs somewhat from that of the seed itself, and its length and breadth are slightly greater. The longitudinal ridges are also less prominent. Here and there, examples (Pl. VI, Figs. 5 and 6) may be found, which appear to show that the outer investment or cupule may have been lobed, though at this stage of development the lobes remained appressed to the seed, and were not

[1] Arber ('05[1]).
[2] Stur ('77), p. 151, Pl. VIII (XXV), Figs. 5–7, and Text-fig. 27 on p. 158.
[3] Oliver and Scott ('04), p. 217, Text-fig. 2.

reflexed. On the whole, so far as one may arrive at a provisional conclusion, it seems probable that these seeds were enclosed in cupules, not unlike those of *Lagenostoma Lomaxi* in some respects, but perhaps having a closer resemblance to certain other fossils, which may now be reviewed in this connexion.

Since the discovery of the seed of *Lyginodendron* it has been generally agreed that certain organs borne on Palaeozoic fern-like fronds, such as *Calymmatotheca Stangeri*, Stur [1], and possibly *Zeilleria delicatula* (Sternb.)[2], whose nature had hitherto remained obscure, are in all probability persistent cupules from which the seeds have fallen. The fertile fronds of the latter species are very closely similar to that described here. The cupules are even smaller, and, at the same time, appear to have been more deeply cleft than in the present instance. But this latter feature no doubt depends to some extent on the state of maturity of the frond at the time when fossilization took place.

Again in *Calymmatotheca Frenzli*, Stur [3], we have in all probability a fertile frond with cupules still in continuity, agreeing fairly closely in habit with the present specimen, and with cupules of about the same size, cleft at the apex into several lobes, as is apparently the case here.

The remarkably small size of the seeds may raise the question whether these organs are really of that nature. The smallest *Lagenostoma* (*L. ovoides*, Will.) known in the petrified state has a length of 4·5 mm. In the case of *L. Sinclairi*, Kidst. MS., a species founded on an impression, the length is 4–5·5 mm.[4] Most of the other seeds, suspected of being of Pteridospermous affinity, are much larger. On the other hand the seed, *Gnetopsis elliptica*, Ren. and Zeill., has a length of 2 mm. There does not appear to me to be any valid objection to the present conclusion, merely on the score of size, for a large number of species of the frond genus *Sphenopteris* are known in which the segments of the pinnules are, as in the present instance, very small indeed, and one would naturally expect to find, in such cases as may prove to belong to the Pteridosperms, some correspondence between the size of the seeds and the pinnules which bore them. Again these fructifications have all the characters of a seed on a small scale (cf. Pl. VI, Figs. 2 and 3), and do not in the least resemble any known Palaeozoic sporangia or synangia. Further, the possibility, by no means slight, that the seeds are invested in cupules, strengthens this conclusion.

[1] Stur, ibid., see also Oliver ('05), p. 412, Text-fig. 6.

[2] Kidston ('84), p. 590, Pl. XXV. It seems to me conceivable that the specimen figured by Kidston, Pl. XXV, Fig. 2, and described (p. 598) as showing 'closed globular involucres,' representing 'the fruit in an early state of development' may, judging by the figure, be a case in which the seeds are still enclosed in their cupules. The general similarity of the specimen to that figured here is striking.

[3] Stur ('85), p. 268, Pl. XXXVIII, Fig. 3, Pl. XXXVII, Figs. 2–3, and Text-fig. 42 on p. 239.

[4] Arber ('05[1]), p. 252.

The next point to be considered is the identity of the seed. In some respects this organ agrees closely with *Lagenostoma ovoides*, Will., but it is on a very much smaller scale. I have not however been able, owing to the minute size of the specimens, to ascertain the characters of the apex in the few instances in which the seed appears to be free from the cupule. Further, it must always be difficult to correlate a seed, occurring as an impression, with one in which the structure is preserved. I do not therefore feel justified, on the present evidence, in referring it to the genus *Lagenostoma*, though I think it probable that it may eventually prove to be nearly related. As it seems advisable to adopt some name for the present specimen, I propose to include the seeds temporarily in the non-committal genus *Carpolithus*, a term already applied to certain seeds of this type, whose affinities are uncertain. As regards the specific name, I would designate it *C. Nathorsti*, sp. nova, in honour of my friend Prof. Nathorst, of Stockholm, who has done so much to increase our knowledge of fossil plants.

The Sphenopterid frond described here does not agree exactly with any species with which I am acquainted. It is however similar to the fertile fronds of *Zeilleria delicatula* (Sternb.), and *Calymmatotheca Frenzli*, Stur. I doubt whether it could be determined specifically, in the present instance, for the lamina appears to be much reduced. I do not therefore propose to designate it other than generically.

Conclusions.

The interest of the present specimen lies in the fact that we have here the very rare instance of a female frond, undoubtedly of the Sphenopterid type, bearing small seeds, probably enclosed in cupules. The inferences are all in favour of the inclusion of this fossil within the Lyginodendreae, although it is impossible, at present, to ascertain whether the seed in question was a true *Lagenostoma*, or a member of some other nearly-related genus. The small size of the seeds themselves, though remarkable, is not inexplicable when we remember that the pinnules of many of the Sphenopterid fronds, which at present rest under the suspicion of being the foliage of Pteridosperms, rather than members of the Primofilices [1], have often quite minute segments or lobes.

Further, in *Carpolithus Nathorsti* we have the first instance where seeds, probably enclosed in cupules, have been found attached to a Sphenopterid frond, the lamina of which can be recognized. Thus perhaps the greatest point of interest of this specimen lies in the fact that the provisional conclusions [2], previously drawn from the evidence of *Lagenostoma*

[1] Arber ('06), p. 218.

[2] Oliver and Scott ('04), p. 229. Arber ('05[1]), p. 258; Arber ('05[2]).

Lomaxi, Will., *L. Kidstoni*, Arber, and *L. Sinclairi*, *Kidst.* MS., as to the habit of the fertile fronds, receives strong confirmation if from an indirect source. That the Lyginodendreae, in common with all the other known members of the class Pteridospermeae, were characterized by an absence of any aggregation of either the male or female organs into cones or stroboli, is a fact of great importance, in view of the strobilate descendants which were probably derived from them in Mesozoic times.

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EXPLANATION OF PLATE VI.

Illustrating Mr. Arber's paper on *Carpolithus Nathorsti*, sp. nova.

(Drawings by Miss G. M. Woodward.)

Fig. 1. Impression of 3 pinnae of a Sphenopteris frond, bearing a large number of seeds, many still in continuity. × 3.

Fig. 2. Detached seed. × 20.

Fig. 3. Seed attached to a lobe which has become broken away from the rest of the pinnule. × 20.

Fig. 4. Pinnule showing several seeds in continuity, probably still enclosed in their cupules. × 7.

Fig. 5. Seeds apparently enclosed in cupules, and in continuity with lobes of the pinnules. × 7.

Fig. 6. Seeds apparently enclosed in cupules. × 10.

Fig. 7. Two seeds, probably still enclosed in their cupules, borne on the lobes of a pinnule. × 10.

Fig. 8. Seeds apparently enclosed in cupules and borne at the ends of the linear lobes of pinnules. × 8.

G.M. Woodward del

ARBER — CARPOLITHUS NATHORSTI *Sp. nova.*

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Huth, lith et imp

The Anatomy and Morphology of Tmesipteris.

BY

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With Plates VII and VIII and thirteen Figures in the Text.

THE material on which the following investigation is based was kindly sent by Professor Thomas, of Auckland in New Zealand, at the request of Professor Seward; it comprised two forms, differing only slightly in appearance and structure, but separated by Dangeard [1] as two species: *T. tannensis* (Fig. I) and *T. lanceolata* (Fig. II).

My thanks are due to Professor Seward, both for his kindness in obtaining the material for me and for his helpful interest in my work and useful suggestions during its progress.

I. Habitat and Distribution.

Tmesipteris is found living as an epiphyte on tree-ferns in New Zealand, Australia, and Polynesia.[2] Each plant consists of an aerial portion and a rhizome, or subterranean region, but has no roots. In my specimens the aerial part varied from three to eight inches in length. It is very difficult to extricate any but small pieces of the rhizome from the tree-fern roots with which it intertwines.[3] I therefore received only small broken posterior portions of the rhizome and short lengths of the anterior region, the latter being attached to the aerial shoots.[4]

Our knowledge of *Tmesipteris* is based entirely on the adult plant: spores have never been germinated, so nothing is known of the gametophyte.

It seems probable that the plant has a saprophytic mode of life, and the occurrence of a fungus[5] growing in the cortical cells of the rhizome supports this suggestion.

[1] Dangeard, Le Botaniste, ii, p. 216.
[2] Baker, Fern Allies, 29-30.
[3] Dangeard, Le Botaniste, ii, p. 168.
[4] Jennings and Hall, 1891, Pl. I, Fig. 4.
[5] Dangeard, Le Botaniste, ii, pp. 223-30, Pl. IX, Figs. 15, 16.

II. External Features.

(1) *Vegetative regions.* The underground portion of *Tmesipteris* runs more or less horizontally and forks at intervals. Generally one of the branches thus formed remains small, while the other continues as the main axis. Rhizome apices were very rare in my material; they are easily distinguished by their smoothness, while the rest of the rhizome is covered

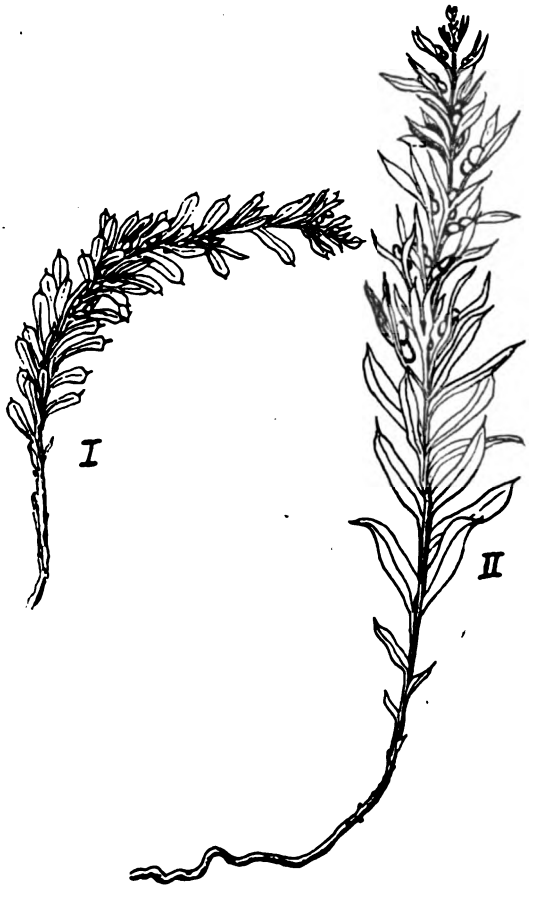

Figure I. *T. tannensis.* Figure II. *T. lanceolata* (⅓ nat. size).

with soft hairs. One apex was divided into three lobes, but investigation of the vascular structure revealed no branching of the stele, and I can offer no explanation of this unusual case.

In every specimen examined the anterior end of the rhizome is directly transformed into a single aerial shoot, but Dangeard describes plants

in which several aerial stems arose from one rhizome.[1] Usually the winding, hairy, and irregular rhizome passes by a more or less straight and glabrous transition region into the upright stem. The surface of the upper part of the transition region becomes gradually ridged owing to the fact that the leaf bases are decurrent. At this stage a number of small scale-leaves, varying from two to six, according to the size of the plant, and reminding one of the leaves of *Psilotum*, are given off. Every transition is found between these and normal leaves. The latter in *T. tannensis* are from a half to three-quarters of an inch long, while in *T. lanceolata* they may reach one and a quarter inches in length. In the former species the largest leaves are found about half way up the stem, and decrease in size again towards the apex of the plant. Each leaf is sessile and ovate—lanceolate, has a mucronate tip, and is adnate to the stem by its base and part of one side. In *T. lanceolata* the largest leaves are found lower down on the stem than in *T. tannensis*; they have no mucronate tip, but taper gradually to a point, and they are lanceolate in shape. It is doubtful whether these two kinds of leaves are sufficiently distinct to warrant in themselves the distinction of the forms bearing them as two species; especially as in some cases[2] both kinds have been found on the same plant. There appears to be no other important difference between the so-called species.

The leaves are arranged spirally in two to five irregular rows, and are vertically placed, like the phyllodes of *Acacia*.

(2) *Fertile region.* In *T. tannensis* the first branches are formed at the region of maximum development of the leaves, in *T. lanceolata* rather above this region. There may be a leaf here and there among the branches, but generally the purely foliar regions of the stem are sharply marked off from the branch-bearing regions, somewhat in the same manner as was noted by Bower in *Lycopodium Selago*.[3] In several cases these regions were found to alternate, six to eight sterile leaves being placed between two fertile regions. No sterile branches such as those described by Bertrand[4] and Dangeard[5] were borne on my specimens, but every branch was fertile and generally bore two leaves and a bisporangiate synangium.

In some plants growth had stopped, and in these cases the main axis ended in a fertile branch or sporophyll, bearing as usual two leaves and a synangium. Presumably the apex is here employed in the formation of the terminal synangium. In one case the last organ borne by the mature plant was a leaf. Other specimens were still growing at the time

[1] Dangeard, Le Bot., ii, p. 168.
[2] Vaughan Jennings and Hall, 1891.
[3] Bower, 1894, p. 353.
[4] Bertrand, Figs. 203, 243–6.
[5] Dangeard, Le Bot., ii, p. 181, Pl. XII, Fig. 5, and Pl. XIV, Fig. 8.

of preservation, and all these bore at their apex numerous fertile branches in all stages of development (Text-fig. II, p. 64). Such plants do not appear to have been among Bertrand's or Dangeard's material, but are figured by Jennings.[1]

Each 'sporophyll' consists of a short axis at the apex of which two leaves and a synangium are situated. The synangium is borne on a short pedicel or sporangiophore, which may, however, in unusual cases become much elongated, and even attain a considerable length.[2] The leaves are exactly similar to those on the main axis. The synangia are generally bisporangiate, consisting of two lobes, each dehiscing longitudinally, and separated from each other by a plate of sterile tissue. A few abnormal fertile branches are described below.

III. Short Summary of Previous Work on Tmesipteris.

The most exhaustive account of *Tmesipteris* is found in Bertrand's monograph[3], which deals very fully with both the anatomy and the morphology. Dangeard[4] has given a more concise description of the genus, of which he recognizes several species, differing chiefly in the shape of their leaves and synangia. This author was the first to investigate the rhizome, and he studied to some extent the fungus[5] found in its cells, and considered by him to be a member of the Chytridiaceae. Jennings and Hall[6] have described the anatomy of *Tmesipteris*, but few new facts are to be gleaned from their account.

The results of the above authors may be shortly summarized as follows: *Tmesipteris* is a rootless plant, the underground portion absorbing moisture by means of rhizoids, containing a fungus and having a single concentric stele, which has usually two exarch protoxylem groups. In no case has an endodermis been seen either in the rhizome or in the aerial stem, and the minute structure of the phloem is as yet uninvestigated. The aerial portion of the plant bears leaves and sterile and fertile branches. The branches each bear a pair of leaves, and in the case of the fertile branches each has also a two-lobed spore-producing organ, formed on the end of a short pedicel arising at the point of divergence of the two leaves. The aerial stem also is monostelic, but here the phloem forms a continuous ring, while the xylem is arranged in several mesarch groups. Bertrand states that the leaves and branches each receive a single trace with a single protoxylem group, but Dangeard describes the formation of diarch steles to supply the branches. Each leaf-trace arises from one of the xylem groups of the stem by division at or near the place of origin, but each

[1] Jennings and Hall, 1891, Pl. I, Fig. II.
[2] Thomas, 1902, p. 343.
[3] Bertrand, Recherches sur les Témsiptéridées.
[4] Dangeard, Le Botaniste, ii, pp. 163-219, Pl. IX-XV.
[5] Dangeard, Le Botaniste, ii, p. 222-30.
[6] Jennings and Hall, 1891.

branch trace is formed by the passing out of a whole group from the stem, its place being subsequently taken in the stem by a group formed by division from one of the others.

The various authors agree that in each fertile branch three bundles are finally found, one of which supplies the spore-producing organ, while the other two form the traces of the two leaves. Dangeard described the forking of the bundle supplying the synangium, but does not appear to have carefully traced its course.

Both the rhizome and aerial stem are said to grow from a single apical cell.[1]

Some abnormal fertile branches have been described by Thomas[2], and the development of the spores has been studied by Bower[3]. The whole question of the morphology of the fertile branch in *Tmesipteris* is one of considerable difficulty, and its discussion will be reserved for the final section of this paper. It must suffice here to state that general opinion regards the 'synangium' as a septate sporangium.

IV. Internal Anatomy.

(*a*) The rhizome grows by a single apical cell in the ordinary manner. Cross-sections very near the tip of the rhizome show the following structure (Fig. 1, Pl. VII):—

The epidermal layer is thin-walled, and some of its cells are prolonged into rhizoids. The cortex is composed of from seven to twelve layers, among which three zones can often be distinguished; the outer and inner zones being composed of starch-containing, and the middle of fungus-containing, cells.[3] The innermost layer but one of the cortex is impregnated with a brown substance which is not soluble in phloroglucin.[3] There is a well-marked endodermis, the cells of which have characteristic thickenings on their radial walls. Inside the endodermis are four or five layers of elongated thin-walled cells which show no marked differentiation into pericycle and phloem. In the centre of the stele are from two to five tracheides, none of which is clearly marked off as protoxylem. Longitudinal sections show that all these tracheides are scalariform, including even the single, youngest, hardly lignified element at the apex. At this stage there are no fibres in the phloem and none of its elements are lignified. Each of the cells of the phloem is elongated, with pointed ends, has pitted areas on its lateral walls, and contains a large granular mass which gives proteid stains and is probably the nucleus.

If a series of cross-sections be cut, it is found that as one leaves the apex of the rhizome the tracheides increase in number and the stele becomes bipolar with two exarch protoxylem groups, each of which is

[1] Thomas, 1902. [2] Bower, 1894 and 1903; Jennings and Hall, 1891.
[3] Dangeard, Le Bot., ii, p. 223; Miss Ford, 1904.

composed of narrow scalariform tracheides, united by large scalariform metaxylem elements. The endodermis is still distinct, and the brown material increases in amount. A few specimens showed fibres in the phloem at this stage, but the ordinary phloem-cells are unlignified.

In some examples monopodial branching took place near the apex of the rhizome, but this was rather an unusual occurrence. Branching is frequent in the middle portion of the rhizome and is generally monopodial, but one case was examined in which both limbs appear equal, and this may be true dichotomy. In this case (Text-fig. III, E, F), just before the forking of the rhizome, the stele divided into two equal parts, each containing one protoxylem group, after which each of the protoxylem groups divided again, restoring the bi-polar structure to both steles. Generally (Text-fig. III, A–D), at the formation of a branch, only one protoxylem group of the main axis divides in order to give rise to the stele for the branch, while the other remains unaffected.

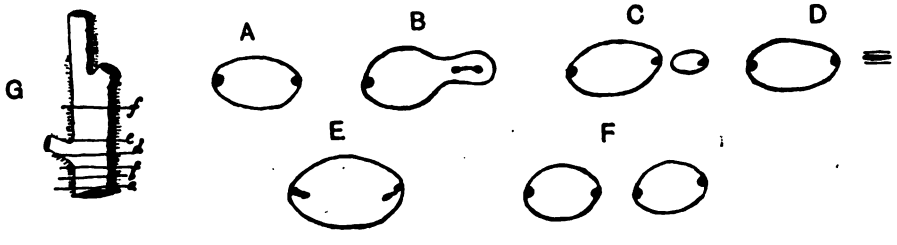

FIGURE III. Illustrating equal and unequal branching of rhizome; = protoxylem. *A–D* = successive stages in the unequal division of the xylem. *E–F* = successive stages in the equal division of the xylem. *G* = portion of rhizome; *a–f* = levels at which sections *A–F* were taken.

(*b*) As the *transition region* is approached, the amount of starch in the cortex diminishes and the fungus and absorbing hairs disappear. In one case only a branch was found to be given off during a very early stage of the transition. The changes in the stele during transition are as follows. At first one, and then several, thin-walled, unlignified cells appear in the centre of the xylem (Text-fig. IV, A), and soon these communicate with the phloem, the cells of which they strongly resemble (Text-fig. IV, B). The xylem then becomes crescent-shaped, and meanwhile one or both protoxylem groups have divided. No distinctly triarch stage, such as that described by Boodle in *Psilotum*[1] is present. Each protoxylem group gradually becomes mesarch.

The metaxylem is then broken across and two distinct groups are formed (Text-fig. IV, C); sometimes these unite again and form a crescent or ring-shaped mass of xylem, surrounding a large amount of thin-walled tissue (IV, D), and reminding the observer of a reduced solenostele in which

[1] L. A. Boodle, 1904.

no internal endodermis or phloem is present. At this stage there is a great increase in the number of tracheides, and sometimes the arrangement of a row of tracheides suggests secondary thickening, but is more probably due to long continued primary growth. Very soon the pith and phloem cells become collenchymatous; it is interesting to note how simultaneously this change takes place in the two tissues.

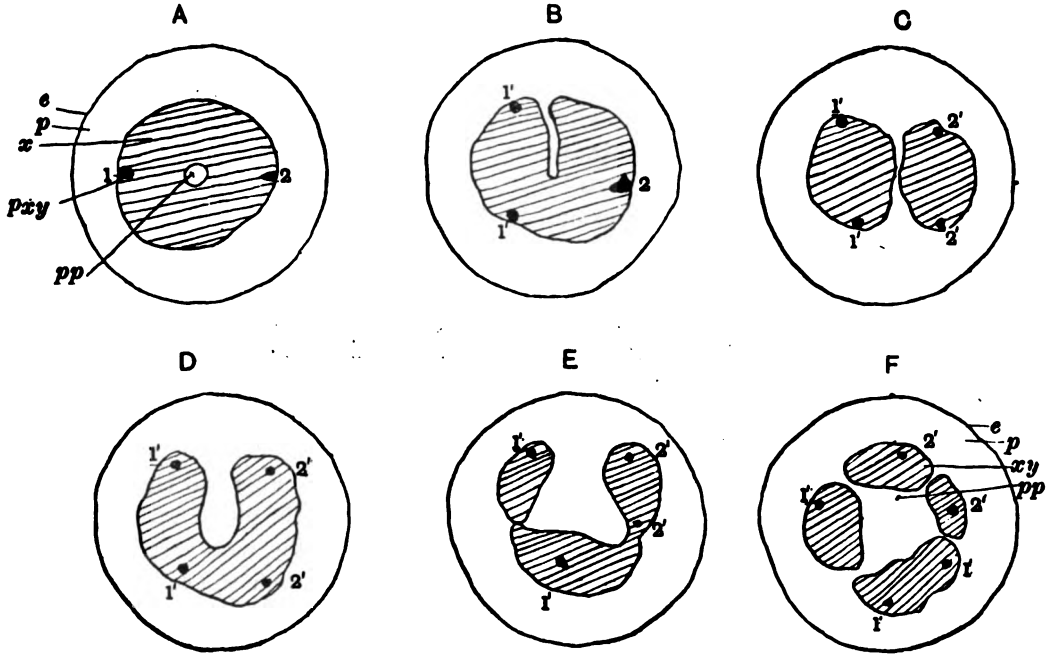

FIGURE IV. *A–F*, illustrating the changes which take place in the stele during transition. *A* = typical rhizome structure; *F* = typical stem structure, below the first leaf; *B–E* = transition region. (*e* = endodermis, *p* = phloem, *x* = xylem, *pxy* = protoxylem, *pp* = pith, 1 and 2 = the protoxylem groups of the rhizome stele; 1 gives rise to 1′, 1′, 1′ in the stem stele, and 2 gives rise to 2′, 2′ in the stem stele.)

(*c*) *The Aerial Stem.* Gradually (Text-fig. IV, F) five distinct groups of protoxylem are formed, sometimes remaining separate, sometimes partly connected by metaxylem. Until now the endodermis has been quite clear, but here it loses its distinctive markings, and its existence becomes hypothetical. Several scale-leaves are given off from the lower part of the stem or from the uppermost part of the transition region, but these receive no vascular supply. At the base of the larger and later of these, elongated thick-walled cells are found, which are continuous with similar cells in the cortex of the stem, and probably serve as conducting elements. The last scale-like leaf receives a very small trace (Figs. 2 and 3, Pl. VII).

A cross-section taken from the lower part of the stem of *Tmesipteris*

has the following structure (Fig. 2, Pl. VII). The epidermis is thin-walled and has no stomata; the cortex is collenchymatous, and its innermost layer is impregnated with a brown substance; no clear endodermis can be distinguished, but the continuous layer of phloem is separated from the cortex by some thin-walled, non-pitted, cells, which include perhaps both endodermis and pericycle. The phloem is itself made up of elongated elements, the walls of which are partly lignified and are scattered with numerous lateral sieve plates. On staining with Iodine Green and Eosin, the general wall of a sieve-tube stains green, and the bright pink sieve-areas appear very prominent. This curious lignification of the sieve-tubes in *Tmesipteris* may be compared with that described in *Helianthus*[1], and with the sclerosed sieve tubes of *Loxsoma*[2], Schizaeaceae[3], Gleicheniaceae and *Trichomanes Prieurii*[4]. It was early observed by Russow[5] in *Tmesipteris*, but his statement was negatived by Jennings and Hall[6]. Internal to the phloem, a ring of five or six mesarch xylem strands surrounds the so-called pith. Each strand is composed of two or three half lignified, finely scalariform, protoxylem elements, enclosed by broadly scalariform metaxylem tracheides. This arrangement of separate mesarch xylem strands, surrounded by a continuous layer of phloem and enclosing a parenchymatous pith, recalls the structure of *Osmunda* and *Todea*. The elongated pith-cells are pitted on their tangential walls, and are often collenchymatous; they contain proteid masses such as were above described in the phloem. Jennings and Hall call these elements sieve parenchyma because of their profusely pitted walls.[6]

The course of the xylem strands is generally straight, with very little anastomosis in the lower part of the stem, but after we reach the leaf-trace region we find a good deal of irregular fusion taking place between them.

(*d*) *Origin of Leaf-Trace.* The ordinary *leaf-trace* arises in the following manner. One of the protoxylem strands of the stem divides about half a centimetre below the departure of the leaf-trace. The outer of the two products of division becomes separated from the inner, and gradually forms an independent group, enclosed by metaxylem. This group projects more and more into the phloem and finally separates from the xylem, becoming in its passage outwards surrounded by phloem, pericycle, and a layer of brown cells (Text-fig. VI, lt_3). Thus it will be seen that the leaf-trace leaves as a rule no leaf-gap. The first leaf-trace often differs slightly in its formation from the later ones; the division of the protoxylem of the group which will give rise to it takes place rather sooner than is usual, and the xylem of the trace is then nipped off and continues for some time as a separate group in the stele.

[1] Boodle, 1902. [2] Gwynne Vaughan, 1901, pp. 84-5, Pl. III, Fig. 10.
[3] Boodle, 1901, pp. 400, 417, Pl. XX, Fig. 19; xxi, Fig. 46.
[4] Boodle, 1901, p. 714, and Poirault, l.c., p. 190, 195.
[5] Russow, l.c., p. 132. [6] Jennings and Hall, 1891.

(*e*) *Origin of Branch-Trace.* The ordinary *branch* or '*sporophyll*' *trace* is described by Bertrand[1] as arising by the passing out of a whole xylem group from the stele, the normal number of xylem-groups being then restored by the division of one of the remaining groups into two. Dangeard, however, does not discuss the origin of leaf- and branch-traces at

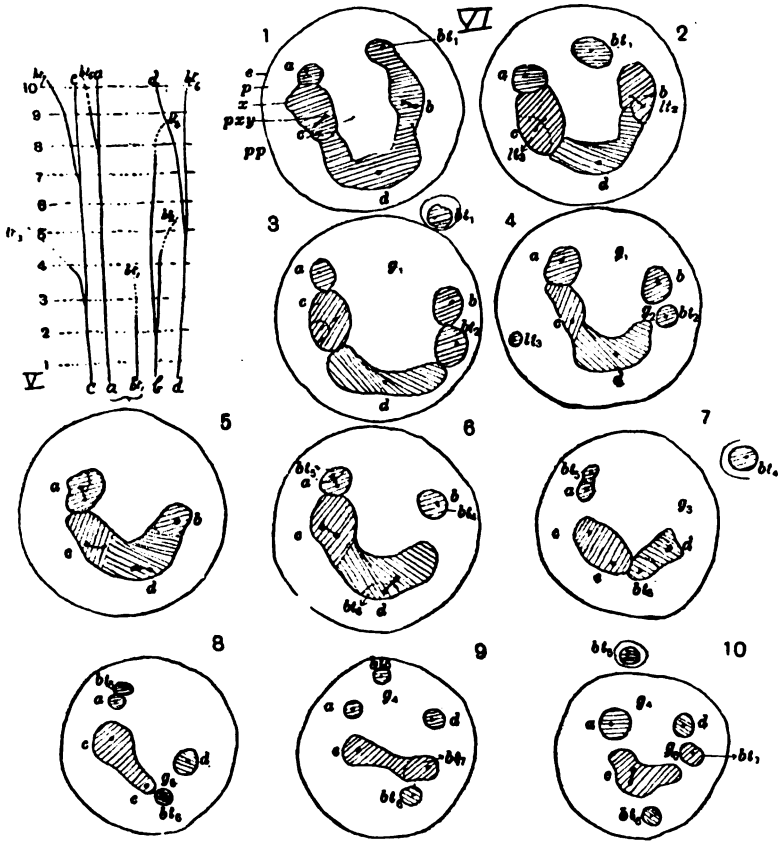

FIGURE V illustrates the course of the xylem strands in the aerial stem, and is constructed from the series of cross-sections shown in Text-fig. VI, 1–10, and taken at levels 1–10 in Text-fig. V. *a*, *b*, *c*, *d* = the xylem bundles, named in the order in which they give off traces. bt_1, bt_2, lt_2, bt_4, bt_5, bt_6, bt_7, bt_8, bt_9, bt_{10} = the traces given off; *bt* = branch-trace, *lt* = leaf-trace; the dotted portion of any trace is that outside the stem stele.

FIGURE VI, 1–10, illustrates a series of cross-sections of the aerial stem through a region bearing one leaf and three fertile branches. Other branch-traces are also seen arising, and will supply branches at a higher level. (*g* = gap, other letters as in Text-figs. IV and V.) In stages 1–5 four xylem groups are present, but in stage 10 only three groups can be seen. The reduction in number has been brought about by both products of the division of Group *b* having passed out as branch-traces. This can also be more clearly understood in Text-fig. V. (Text-fig. VI is not drawn perfectly to scale.)

any length, but asserts the similarity of the two, the chief difference consisting in the greater number of elements employed in the formation of the

[1] Bertrand, l.c., pp. 256–7.

latter.[1] It can be shown that the processes involved are essentially similar. The division of the protoxylem group, which is the first step in the formation of the branch-trace, takes place about two centimetres below the departure of the latter from the stem. The strand of xylem which is to pass out as a branch-trace, is soon nipped off, and runs for a considerable distance as a separate constituent of the stem stele, seemingly forming an additional strand (Text-figs. V and VI, bt_1, bt_2). It then passes out, becoming in the ordinary way enclosed by phloem, and leaves a gap (Text-fig. VI, *g*) behind it in the ring of xylem strands. Hence the apparent difference in the appearance presented at the point of departure of leaf- and branch-traces is that in the former case a strand of xylem is left in the stele exactly opposite the trace, while in the latter there is a gap in that position. Yet the only real cause of this difference is that in the one case the division of the strand to form the trace takes place earlier than in the other.

The lower fertile branches seem to receive their traces in the same way as the leaves, or, at any rate, less time elapses between the formation and departure than is required by the later branch-traces. Almost immediately after a branch-trace has gone off, or sometimes even before, another of the xylem groups divides to form a new branch-trace which remains for some time in the stele and brings the number of strands up to the original number (Text-fig. VI, 7 *c*, bt_7). In one or two cases both products of the division of a group were seen to pass out as branch-traces fairly low down in the stem (Text-figs. V, VI, *b*, bt_2 and bt_4); always, as the apex is approached and the xylem groups become confluent, obliterating the pith, one group after another behaves in this manner, the number thus being gradually reduced, until finally only one group is left. Here in one series of sections four leaves occurred, and their leaf-traces were formed in the ordinary manner by the splitting off of a strand of xylem and its enclosure by phloem and subsequent rapid departure from the stele. In this same series there was next formed a branch, its trace being of necessity also produced by a similar division of the only remaining group. This division took place longer before the departure of the trace than was the case in the formation of the four leaf-traces just mentioned. Finally a branch was formed which limited the growth of the main axis (Text-fig. VII). As described above,[2] a synangium is usually borne

FIGURE VII. Apex of an adult specimen, showing a fertile 'branch', one limb of which is branched again.

[1] Dangeard, l. c., p. 179.

[2] Ibid., pp. 65–6.

on a branch with one leaf on each side, but here a synangium was formed at the termination of the main axis, there being one leaf on one side, but a second normal fertile branch in the corresponding position on the other side.

A minute investigation of the structure of the stem near the apex (Fig. 4, *a*, *b*, *c*, Pl. VII) shows that the cortex is here composed of only five or six layers of cells, the innermost of which does not in this region form the brown substance. The phloem often contains fibres, and is already lignified. The xylem forms a solid mass of scalariform elements with one group of protoxylem, which is also scalariform even to the last terminal element. In plants preserved while still growing in length a single apical cell can be made out with some difficulty.[1]

(*f*) *Leaves.* The leaves of the two forms are similar in structure, the chief difference consisting in the shape of their cross-section. In both species the little scale-leaves remind the observer of *Psilotum*. The lowest of these consist simply of a small mass of mesophyll, enclosed in a layer of epidermis. Mesophyll, epidermis, and stomata are of the characteristic nature described below for normal leaves.

The ordinary leaf of *T. elongata* has stomata on both sides, but that of *T. tannensis* has stomata only on the adaxial side. The stomata are of the ordinary type, with two guard-cells and no accessory cells. Their development in the young leaf is of no especial interest; they arise from an epidermal cell in the simplest manner by division into two.

The epidermal cells of the adult leaf are large and stellate; their walls are thickened in bands (Fig. 6, Pl. VIII), and when stained with Iodine Green and Eosin the bands stain green, while the areas in between them become pink. The mesophyll is composed of curious lobed cells (Fig. 5, Pl. VIII), quite similar to those of *Psilotum*.[2] A single vascular bundle runs in an almost vertical direction through the cortex of the stem, traverses the centre of the leaf, and terminates in a single tracheide just below the mucronate tip. The bundle during the greater part of its course consists of four or five scalariform tracheides surrounding one or two narrowly scalariform protoxylem elements, and enclosed by lignified phloem and a more or less evident layer, which probably represents the endodermis.

The base of the leaf is decurrent, and thus a cross-section of the leaf-bearing part of the stem shows one or more protuberances due to leaf bases, in which the lobed mesophyll is always conspicuous.

(*g*) *Fertile branches.* In this description I can only include the fertile branches, but the resemblance between my sections of these and Bertrand's figures of sections through a sterile branch[3] is most striking, and there seems some doubt whether sterile branches with no synangial rudiment are ever found.[4]

[1] See Jennings and Hall, 1891, Pl. IV, Figs. 27, 28; Pl. III, Fig. 18.
[2] Ford, 1904. [3] Bertrand, l. c., Figs. 243–246. [4] Thomas, 1902.

A single vascular bundle, very like a leaf-trace in appearance but of rather greater bulk, enters the branch axis. In all the cases examined only one protoxylem group is present, but Bertrand[1] describes cases in which the bundle was diarch. The single bundle very soon branches into

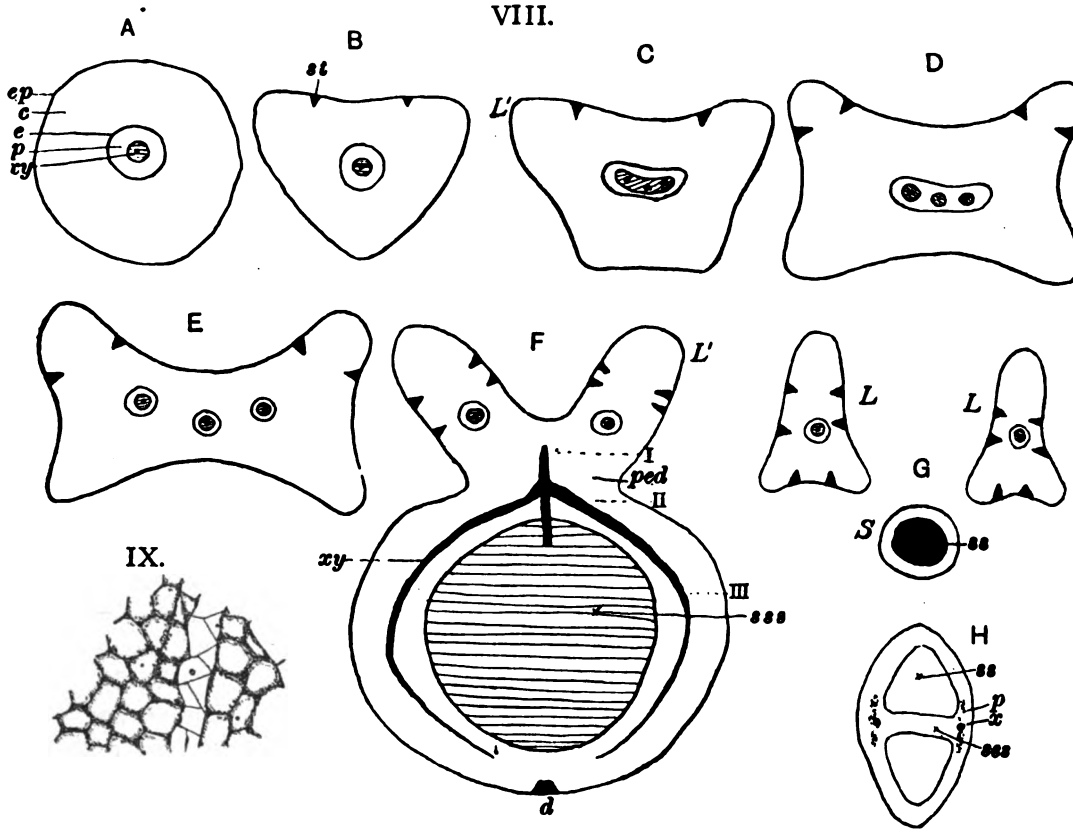

FIGURE VIII. *A–G*, illustrating a series of cross-sections through a fertile branch. *A–E*, through stalk of branch; *F*, through origin of two leaves and synangium; *G*, through two leaves and apex of synangium; *H*, tangential section through synangium at level III, showing phloem extending up the walls of the synangium.

FIGURE IX. Surface view of line of dehiscence of synangium showing layer of thin-walled cells; *ep* = epidermis; *c* = cortex; *e* = endodermis; *p* = phloem; *xy* = xylem; *st* = stomata; *ped* = synangium pedicel; *sss* = synangial septum; *S* = synangium; *ss* = spore cavity; *d* = line of dehiscence; *L* = leaves; *L'* = decurrent base of leaves; *I*, *II*, *III* = levels in *F* at which Figs. 10, 11, 12, Pl. VIII, were taken.

three (cf. Text-fig. VIII, D, and Fig. 7, Pl. VIII), and the three products of division move farther apart and become each surrounded by a separate 'endodermal' layer. Meanwhile the cross-sections, which were circular before, become more or less square, and soon two of the corners of the square begin to project. These corners correspond to the ridges formed by

[1] Bertrand, pp. 256 and 272.

the decurrent bases of the two leaves; stomata are present on both sides of the ridges, and are occasionally found on other parts of the axis. In between the two less projecting corners the synangium pedicel arises, and one of the three vascular bundles passes into this structure, while the other two are each continued into one of the two leaves. The structure of the leaves corresponds in every respect with that of the leaves of the main axis.

The synangium itself is composed of two lobes, each containing spores,[1] and separated from each other by a plate of sterile tissue. The outer wall of the synangium consists of a single layer of cells with thickened walls. Dehiscence takes place along a double line of thin-walled cells, which traverses each lobe in a longitudinal direction (see Text-fig. IX, p. 74). Under the thick epidermal layer are two or three layers of ordinary thin-walled pitted parenchyma, which enclose the cavities containing the spores. Each of the pits is sometimes surrounded by a lignified ring. There is no definite tapetum; the spores are formed in tetrads and are bilateral and oval in shape, resembling closely those of *Psilotum*.

The pedicel of the synangium is very short, and is continuous with the plate of sterile tissue which separates the two lobes. Its vascular bundle terminates in this plate, and may end just on the edge of it (Fig. 8, Pl. VIII), or may run for a short distance across it (Text-fig. VIII, F, p. 74). In either case it gives off, just as it enters the synangium, a branch on either side, which run in opposite directions round the periphery of the plate, and sometimes meet on the other side, thus forming a complete ring. All the xylem of the synangial trace is contained in this ring, and the cells of the plate are also found to be lignified to a considerable extent. The phloem constituent of the synangial vascular supply is much greater in bulk than the xylem, and extends for a short distance up the walls of the spore cavities (Text-fig. VIII, H, p. 74). It is composed of rather short sieve-tubes with lignified walls. A series of tangential sections through the synangium shows clearly (1) the single bundle in the pedicel (Fig. 9, Pl. VIII); (2) the division of this bundle into three (Fig. 10, Pl. VIII); (3) the disappearance of the central trace and wide separation of the two lateral ones on either side of the sterile plate (Fig. 11, Pl. VIII); (4) and finally at the side of the synangium furthest from the axis these also die out, or are represented by a small amount of phloem only.

(*h*) *Abnormal branches.* The material supplied to me contained very few abnormalities in comparison with those described by Thomas [2].

1. One of the plants bore a three-sporangiate synangium near the apex. The main axis gave rise first to a branch bearing this synangium and two leaves, then to a single leaf, and was finally terminated by an ordinary

[1] For development of spores see Jennings and Hall, 1891, Pl. V, Figs. 40–42.

[2] Thomas, 1902.

synangium with two leaves. The position of the odd leaf was rather unexpected; conceptions derived from the usual symmetry would have led me to suppose that it belonged to the branch bearing the abnormal synangium, but its trace clearly arose from the stem stele after the departure

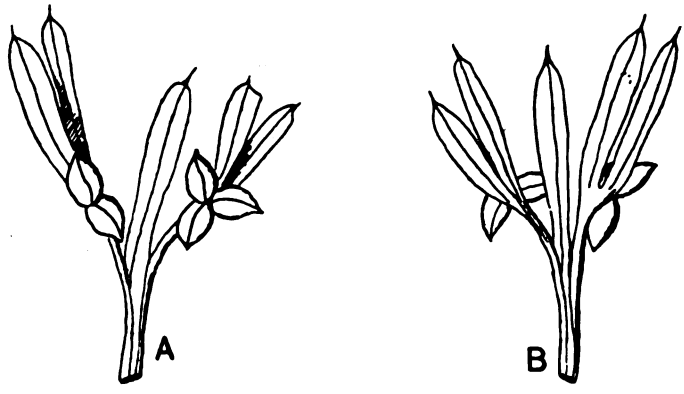

FIGURE X. Apical region of an adult plant, showing a three-sporangiate synangium; *A* = front, *B* = back.

of the branch-trace. The three-sporangiate synangium appeared to receive three bundles from the pedicel trace, but there were difficulties in its

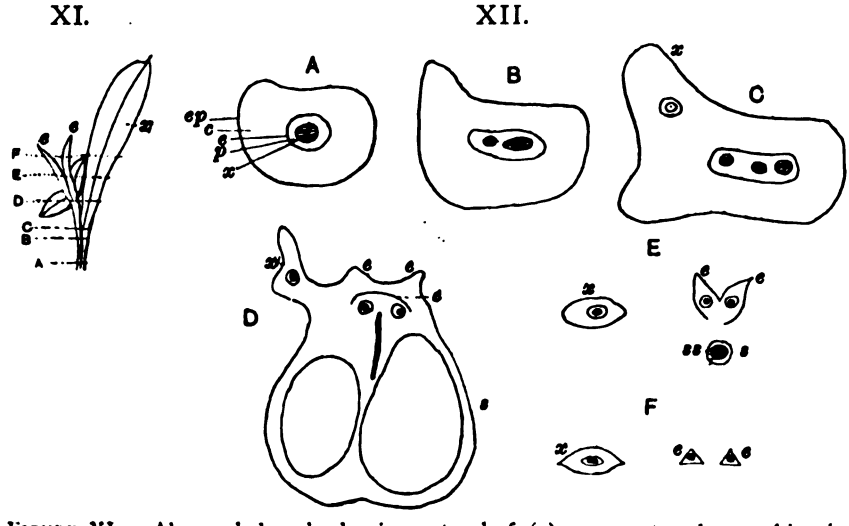

FIGURE XI. Abnormal branch, bearing extra leaf (*x*). *e*, *e* = two leaves of branch, unusually small; *A–E* = level at which sections *A–F*, Text-fig. XII, are taken.

FIGURE XII. *A–F*, series of cross-sections taken at level *A–F* in Text-fig. XI. Letters as in Text-figs. VIII and XI.

investigation, and it is quite possible that a fourth was also present. All the loculi of the synangium contained normal spores.

2. One lateral branch had an extra leaf (Text-fig. XI, *x*). The leaf-

trace supplying this leaf arose from the axial strand of the branch just before it breaks into three in the ordinary manner.

3. Lastly, there was the case mentioned above[1] as occurring at the apex of the plant of which a continuous series of sections was made. In this case a branch, bearing one leaf and a synangium, arose from the main stem, which was then terminated by a normal branch. The stele of the stem branched into three exactly as does usually the stele of a branch; the middle of the three traces supplied the synangium, one of the lateral ones ran into the leaf, while the third became the stele of the normal branch.

(*i*) *Ramular and Foliar Gaps.* Since *Tmesipteris* is one of the larger-leaved members of the Lycopodiaceous series, the vascular supply of its leaves is of interest in relation to the question of leaf-gaps. This interest is increased by the fact that the xylem in this plant has a mesarch structure.

Great stress has been laid by Professor Jeffrey[2] on the absence of leaf-gaps in the Lycopodiaceae as contrasted with the presence of ramular gaps, the absence of leaf-gaps being considered by this author as constituting an essential difference, of real phylogenetic importance, between this phylum and all other vascular plants.

It has been shown above[3] that while the exit of the ordinary leaf-trace from the stele in *Tmesipteris* leaves no gap in the xylem, the exit of the trace supplying the 'sporophyll' generally leaves a large and obvious space. At the same time it has been explained that, though the difference between these two methods of development of traces is at first sight very considerable, in reality it is only a question of degree, and is due to the earlier preparation for the formation of 'sporophyll' than leaf-traces. If we look upon the 'sporophyll' as a foliar organ, we have here a larger leaf than is ordinarily present in the Lycopod series, whose larger vascular supply has caused the formation of a gap in the xylem of the stem, opposite its place of exit. This gap is not essentially a new production, but is mostly due to a difference in degree between the formation of the two kinds of traces.

If, on the other hand, we consider the sporophyll in *Tmesipteris* to be an axial organ, the nature of what may then be looked upon as a ramular gap is still of interest, since it shows us how small is really the difference, at any rate in this plant, involved in the presence and absence of gaps.

It would seem that *Tmesipteris* also throws some light on the meaning of mesarch structure. In all the higher plants it is the 'phanerogamic wood'[4] or centrifugally developed xylem which gives rise to the leaf-traces. In the microphyllous Lycopods the very small and probably reduced

[1] See above, p. 72, and Text-fig. VII. [2] Jeffrey, 1902. [3] See above, pp. 70–1.
[4] Scott, New Phyt., vol. i.

leaf-traces can take their origin from the centripetal xylem in the stem, and do not require any especial provision for their formation. In *Psilotum* there are no leaf-traces, and in *Isoetes* and the Selaginellas the stelar structure is essentially aberrant. But in *Tmesipteris* we have a plant of which the leaves are supplied by traces arising from a stele comprising both centripetal and centrifugal wood, and the latter alone, in addition to the protoxylem, is concerned in the formation of the leaf-traces. The presence of mesarch xylem in the large-leaved cone of *Cheirostrobus*,[1] which belongs probably to a group in which exarch structure is prevalent, also suggests the correlation of mesarch protoxylem with the formation of large leaves.

V. Comparison with Psilotum and Sphenophyllum: General Morphology.

Tmesipteris and *Psilotum* are the only two living members of the Psilotaceae. The two plants are in many respects similar in structure, but *Psilotum*[2] is simpler than *Tmesipteris*. Their rhizomes agree both in appearance and in detailed anatomy, but no bulbils such as those described in *Psilotum*[3] appear to be present in *Tmesipteris*. The difference in the structure of the stems can be easily explained on the theory of reduction in *Psilotum*. The leaves of the latter plant are extremely small and have no vascular supply, so that the greater part of the function of assimilation is performed by the stem. The stele of the stem has a ring of xylem with three to five projecting groups of exarch protoxylem. Inside the xylem is a lignified pith, and outside is a ring of phloem. An endodermis is present in both rhizome and aerial stem, and in the aerial stem the phloem is slightly lignified. A rudimentary kind of secondary thickening has been described in the transition region of *Psilotum*,[4] and one of the chief reasons for the investigation of *Tmesipteris* was the desire to see if anything of a corresponding nature was present in this plant. Nothing of the kind was, however, found in any of the specimens examined.

Boodle[4] supposes that the common parent of the Psilotaceae had an aerial stem with a rayed mesarch xylem mass, and possessed also the power of secondary thickening. The anatomical resemblance between such a plant as this hypothetical ancestor and *Sphenophyllum* is obvious. He suggests that the suppression of the leaf-traces has caused the loss of centrifugal xylem in the one genus, while the influence of the large leaf-traces in the other has broken up the xylem into masses.

It appears also possible that the secondary absence of large leaf-traces in *Psilotum* has caused the fusion of originally separate masses of xylem

[1] Scott, 1897.
[2] Ford, 1904.
[3] Solms-Laubach, 1884.
[4] Boodle, 1904.

into a ring, and this suggestion is supported by the breaks found occasionally by Miss Ford in the xylem ring in *Psilotum*.[1] It seems also fairly easy to explain the loss of secondary thickening in *Tmesipteris*. In both of these slender-stemmed plants only a small amount of mechanical and conducting tissues is required. In some parts of the larger specimens of *Psilotum* the extra needs are supplied by secondary elements, but in *Tmesipteris* the centrifugal xylem, which here, as everywhere, forms the leaf-traces, is sufficient, in addition to the centripetal wood present in both plants, for all requirements. The lignification of the phloem in *Tmesipteris* may also be of mechanical importance.

Again, one might, of course, consider the secondary thickening in *Psilotum* to be an adaptive and not a primitive character. When the centrifugal xylem became no longer necessary for the formation of leaf-traces, it was probably no longer formed. But insufficient conducting elements would then be present in such regions as the bases of branches or the transition region of large plants, and here a power of secondary growth may have arisen. If this last interpretation be the true one, it may be said that in *Psilotum* centrifugal xylem is no longer formed by primary growth, but in certain cases is produced secondarily by a cambium.

The discovery of mesarch structure in the lower parts of some *Psilotum* stems[2] may be supposed to suggest this as an ancestral character.

It has recently become usual to compare the Psilotaceae with Sphenophyllales[3] and to draw conclusions as to relationship from such a comparison. This comparison is based on the following characters:—

1. The rudimentary secondary thickening in *Psilotum*, and the general resemblance of the exarch rayed xylem mass (especially in triarch steles) of that plant, and the similar xylem mass in *Sphenophyllum*.

2. The mesarch xylem of *Tmesipteris*, separated into groups, recalls the structure of *Cheirostrobus*.[4] But anatomical characters such as mesarch protoxylem and a power of secondary growth in thickness[5] are so much a question of habit—the presence of mesarch xylem in particular being commonly correlated with the presence of leaf-traces—that it seems arbitrary to found theories of relationship on them.

3. The real basis of such theories lies therefore on the third comparison made between the two families, a comparison based entirely on prevalent conceptions of the morphological nature of the 'sporophyll' in the Psilotaceae. It will now be necessary to discuss the morphology of that organ.

The interpretation of the structure, both of the whole fertile branch and of the synangium itself, is a problem of much difficulty. The first

[1] Ford, 1904, Pl. XXXIX, Fig. 13. [2] Boodle, 1904.
[3] Scott, Studies, 1900. Scott, 'Cheirostrobus,' Phil. Trans. 1897, p. 27; and Thomas, 1902.
[4] Scott, 1897, p. 417. [5] See above, pp. 78-9.

question is whether the synangium is the homologue of one sporangium or of several. Bower's account of the development of the sporangium in *Tmesipteris* supports the view that it is derived from a single sporangial mass by septation. The tissue of the septum has an origin quite similar to that of the sporogenous tissue, and in abnormally simple forms the septum may be aborted, and the tissue which would have given rise to it may form a tapetum or even give rise to spores. The septation of a sporangium is by no means an unusual phenomenon, for example the anthers of some Onagraceae [1] may be mentioned here. In the Lycopodiaceous series the trabeculae of *Isoetes* and *Lepidodendron* suggest incomplete septation. At first sight the reception of two bundles by the synangium in *Tmesipteris* may appear to imply the separate origin of the lobes; but this is not a necessary conclusion, on the contrary the large masses of developing spores so obviously require a plentiful supply of food and water that the branching of the sporangial trace would seem a very likely occurrence.

On the other hand Bower [2] found that a study of the development of the sporogenous masses in *Psilotum* showed each to be referable to a single parent cell. This state of affairs was not proved in *Tmesipteris*, but, as far as it goes, it supports the view of the separate origin of each lobe of the synangium, and their subsequent fusion. As far as comparative morphology is concerned there is little of a positive nature to support either view. Bower holds that the synangium is derived from an originally simple sporangium like that of a Lycopod, but present opinion appears to incline more to the view that the Lycopodiales are reduced rather than primitive; and it is therefore possible that their sporangium is to be looked upon as formed by the ultimate fusion of such a group of sporangia as we find partially fused in *Tmesipteris*. In the Calamites, in *Cheirostrobus* and in *Sphenophyllum*, there is generally more than one sporangium borne by a sporophyll, and *Sphenophyllum majus*,[3] with its tuft of four sessile sporangia, bears a most striking resemblance to *Tmesipteris*. If the comparative evidence is to be considered as of any value, it seems to lend support to the view that the synangium was originally formed by the fusion of separate sporangia, but it appears to me very difficult to come to any definite conclusion on the matter.

It is otherwise, however, with the theories concerning the morphology of the whole sporophyll.

On this question there have been for some time two principal views. Mettenius, Luerrsen, Celakovsky, Solms Laubach, Dangeard, Bower, Scott, and Thomas have believed the 'sporophyll' to be a foliar organ; whilst Juranyi, Strasburger, Goebel, Sachs, and Bertrand have supposed it to be a fertile branch, the first two leaves of which have the appearance of

[1] Bower, 1896, p. 5. [2] Bower, l.c., 1894. [3] Scott, 1907, p. 152, Fig. 3.

a forked bract. It will be necessary to consider some of these views in greater detail.

Foliar Theory. Dangeard[1] suggests that two leaves have coalesced by their petioles, and have given rise to a synangium at the point where this union ceases. The mere fact that the sporophyll trace has a single and not a double origin appears to me sufficient to dispose of this theory. If we are to consider that two leaves have fused and given rise to a sporophyll, we should certainly expect that their traces, even if not distinctly separate, would at least originate from two separate protoxylem groups of the stem-stele.

The other members of the foliar school suppose the sporophyll to be a single-lobed leaf. Bower[2] 'understands the synangium to be a lateral organ, originally foliar in nature, and an outgrowth from a single lobed leaf'. He thus homologizes the synangial apparatus in *Tmesipteris* with a single Lycopodian sporophyll.

Thomas[3] lays great stress on the presence of green assimilating cells and stomata in the stalk of the sporophyll. But when we take into consideration the fact that the two leaves of the branch have, like all the other leaves of *Tmesipteris*, decurrent bases, it is not surprising to find leaf tissue on the sides of the branch. Also in the nearly allied plant, *Psilotum*, we find assimilating tissues in the stem itself. To support his view Thomas brings forward certain abnormal cases in *Tmesipteris*—sporophylls with repeated dichotomy and two or three synangia; sporophylls with a long stalk instead of a short pedicel; and cases in which the synangium was replaced by a leaf lobe of normal appearance, the whole branch then being comparable with a tri-lobed leaf. The examples of repeated dichotomy generally occurred at the region of maximum development of the plant, and are supposed by Thomas to be dependent on abundant nutrition; but Text-fig. VII, p. 72, also represents a second dichotomy seen near the apex of the plant. It seems to me easier to regard this kind of abnormality as the repeated dichotomy of a branch than as the lobing of a leaf. The occasional presence of a long stalk to the synangium also suggests that the usually short pedicel is of an axial nature. The third abnormality is of less value, being obviously a monstrosity.[4]

Scott considers the foliar view of the sporophyll in *Tmesipteris* as of great interest in that it throws some light on the relation of that plant to *Sphenophyllum* and *Cheirostrobus*.[5]

Axial Theory. The axial theory of the sporophyll in *Tmesipteris* is too simple to need much exposition, and some of the arguments have been given above. It appears to me that the chief of these is to be found

[1] Dangeard, Le Bot., ii.
[2] Bower, 1894.
[3] Thomas, 1902.
[4] Scott, 1907, p. 139.
[5] Scott, 1897, Proc. Roy. Soc., p. 117, and 1897, Phil. Trans., p. 27, and Studies, 1900.

in the presence of the third bundle supplying the synangium,[1] which may represent the vascular supply of the apex of the synangial branch. The presence of a third bundle in the sterile branches[2] may perhaps be also of interest in this connexion.

Strasburger[3], in his paper of 1873, considered the axial nature of the sporophyll in the Psilotaceae to be so obvious that he carried his arguments further, and suggested that the sporangium in the Lycopods is also primitively an axial organ which has become associated with the sporophyll in the course of evolution.

It seems very probable that the distinction so generally drawn between foliar and axial organs may prove to be somewhat forced, and it is especially among the lower Pteridophyta that we should expect to find it less sharp; indeed, according to views recently expressed by Tansley[4], we are to look upon a leaf as a specialized branch derived from an ultimate ramification of the primitive thallus. Inasmuch, however, as the general idea of a leaf is concerned, it is certainly a flattened lateral emergence, capable of limited growth, while a branch is a non-flattened emergence which grows apically for a considerably greater period of time. The sporangial branch of *Tmesipteris* appears to belong to the latter of these two categories, and the sporangiophore bearing the synangium seems to be the transformed apex of a branch. Text-fig. VII, p. 72, is a case in which a pedicel with its synangium terminates the main axis; and its position makes it difficult to regard this sporangiophore as foliar, though it might be looked upon as an organ *sui generis*.[5] The close association of the sporophylls with the synangium need not necessarily be a primitive character, and it may well have been that a scattered arrangement of the reproductive and assimilating members prevailed in some of the ancestral strobili. There are obvious advantages in a connexion between those vascular elements which bring elaborated food products from the assimilating tissues and those which supply the developing spores. Such a connexion of the vascular systems of bract and sporangiophore is seen in *Calamostachys*,[6] and has been interpreted as indicating that the arrangement in that cone is derived from one in which a sporangiophore was found in the axil of a bract, as in *Palaeostachya*. But recent unpublished investigations of Mr. Hickling's, quoted by Scott[7], have shown that in *Palaeostachya* the trace supplying the sporangiophore pursues a very remarkable course; while arising from the trace of the bract in the axil of which the sporangiophore is found, it runs vertically for some distance up the stem, and then sharply bends 'back-

[1] See above, pp. 73–4.
[2] Bertrand, l. c., Figs. 243–246.
[3] Strasburger, B. Z., 1873.
[4] Tansley, 1907.
[5] Bower, 1903, p. 192.
[6] Renault, 1896, pp. 130–4.
[7] Scott, 1907, pp. 159–60. Note.—Since the above was written, Mr. Hickling's paper has appeared; Ann. of Bot. 1907. See esp. Text Fig. 1.

wards to enter the sporangiophore'. This arrangement certainly suggests that 'their present axillary position is secondary, not primary'.

Finally, in the Sphenophyllales and Psilotales there are no longer any signs of the separate origin of bract and sporangiophore, and their close association has often been regarded as a matter of course. It appears to me that Lignier's series[1] (*Sphenophyllum Dawsoni*, *Palaeostachya*, *Calamostachys*), which was constructed to illustrate the gradual shifting of the sporangiophore from the sporophyll on to the axis of the cone, may with equal ease be read in the inverse order, and then illustrates the removal of the sporangiophore from the axis on to the sporophyll.

The fossil cone, *Spencerites*[2], perhaps throws some light on the possible origin of the Lycopods by reduction from some more complicated form. The proximal part of the sporophyll in *Spencerites* may represent the remains of a branch axis bearing a single bract and a sporangium on a short stalk. Scott[3] considers it likely that the present Lycopods are reduced rather than primitive, and Strasburger[4] suggested in 1873 that the formation of axillary bulbils, in a position exactly similar to that of sporangia, in *Lycopodium Selago*, is an argument for the theory that the sporangium in this genus is of the nature of a reduced axillary shoot. It seems quite possible that the axis of the original sporangial branch has become much reduced in the Lycopods; according to this view a whole fertile branch in *Tmesipteris* is homologous with a single Lycopodian sporophyll. In conclusion, while, on this view, it is not possible to found a relationship between the Psilotales and Sphenophyllales on the ground of the common foliar nature of their sporophylls, it appears not unlikely that the primitively axial nature of the spore-bearing organs may yet be demonstrated in *Sphenophyllum* and *Cheirostrobus*, and that a relationship between the two families may be finally established. Each so-called 'sporophyll' in the Sphenophyllales may perhaps be proved to be equivalent to a reduced branch bearing sporangia and leaves, the original axis of which has become much reduced during the process of formation of a cone from a lax arrangement of scattered fertile branches, such as is characteristic of the stems of Psilotaceae to-day.

VI. Systematic Position.

The fact that the gametophyte of the Psilotaceae is unknown is a great hindrance in the formation of any conception of their real relationship to other families. The only case of a prothallus possibly belonging to *Psilotum* was recorded by Lang[5], and resembles a Lycopod prothallus of the 'clavatum' type.

The Psilotaceae were formerly considered to be nearly allied to

[1] Lignier, 1903, p. 95.
[2] Berridge, 1905.
[3] Scott, 1907, p. 175.
[4] Strasburger, 1873.
[5] Lang, 1904.

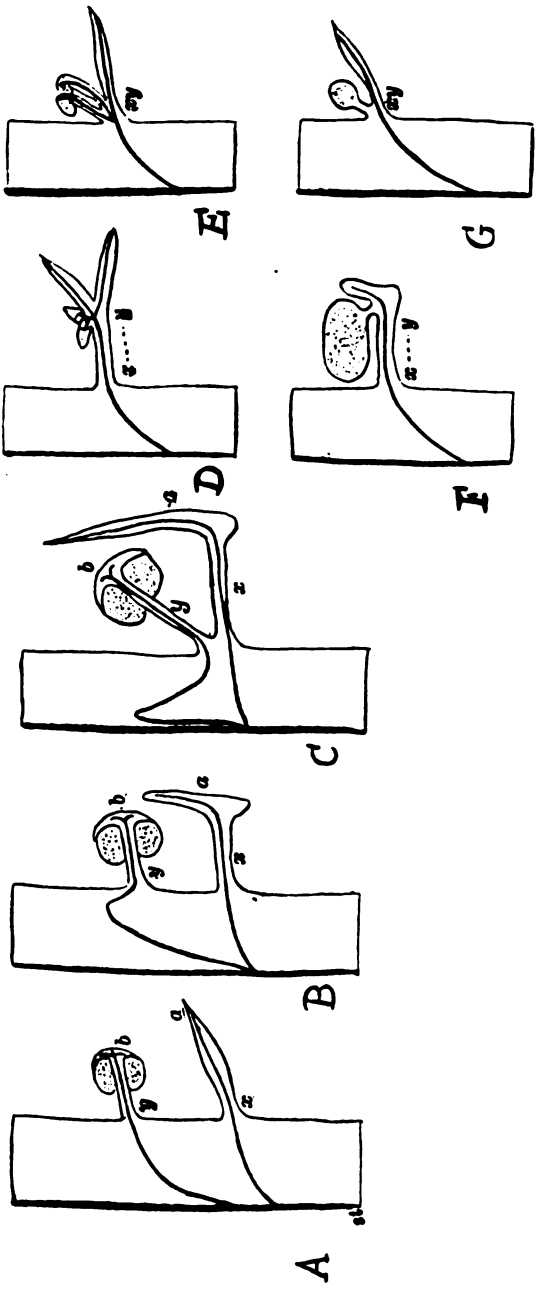

FIGURE XIII. Illustrating morphological relationships discussed in the text. *a* = flattened assimilating appendage; *b* = axial sporangium-bearing appendage; *x* = petiole of *a*; *y* = axis of *b*; *xy* or *x...y* = result of fusion of *x* and *y*, practically reduced to nothing in *Sphenophyllum* and *Lycopodium* (but perhaps present in *S. majus*); *st* = stele of main axis of cone. *A* = hypothetical primitive ancestor, the branch-trace, as in *Tmesipteris*, arises comparatively earlier than the leaf-trace. *B* = *Calamostachys*, branch and leaf-trace arising almost together, as also in *C* = *Palaeostachya* (after Hickling), in which is seen the commencement of association of *a* and *b*. *D* = *Tmesipteris*, *E* = *Sphenophyllum*, *F* = *Spencerites*, *G* = *Lycopodium*; in all four cases, *x* and *y* and the traces of *x* and *y* are more or less associated and fused.

Lycopodium, and were placed by Baker[1] in the Lycopodiaceae, as genera equivalent in value to *Lycopodium*. Campbell[2] still places the order Psilotaceae in the Lycopodiales; Scott[3] prefers to found a group, Psilotales, which is more nearly allied to the Sphenophyllales than to the Lycopodiales; Thomas[4] and Bower[5] have come to the conclusion that the Psilotaceae are sufficiently nearly allied to the Sphenophyllaceae to warrant a position in Sphenophyllales.

It is, perhaps, well to recapitulate here the resemblance noted by Scott and Thomas between the Psilotaceae and Sphenophyllaceae. These are—secondary thickening in *Psilotum* and *Sphenophyllum*; mesarch position of the protoxylem in *Tmesipteris* and *Cheirostrobus*; and the similar morphological nature of the spore-bearing organs in the two families. These authors believe that the synangial 'branch' in *Tmesipteris* is to be compared with a sporophyll of *Sphenophyllum* or *Cheirostrobus*, the synangium with its pedicel being equivalent to the sporangium or sporangia with the sporangiophore, borne by a sporophyll of one of these cones, the leaves of the synangial 'branch' corresponding to the sterile segments of such a 'sporophyll'. They think also that the dichotomous division of the sporophylls in the Psilotaceae, the fact that their sporophylls are more elaborate than the vegetative leaves, the groups of sporangia on a single sporophyll, cases of abnormally branched sporophylls, and 'the normal modification of the ventral leaf-lobe to form the synangium', are all indications of affinity with the Sphenophyllales.

While many of these latter points of comparison are dependent on the authors' interpretation of the morphology of the synangial branch, it has been shown above that others may be explained on the axial theory of the homology of that organ, and the great similarity between the sporophylls of *Tmesipteris* and *Sphenophyllum majus*, both in distribution on the axis and in general appearance, is alone enough to suggest some relationship between the two families. But unless it is possible to look upon the sporophyll in *Sphenophyllum* and *Cheirostrobus* as a sporangiophore, which is axial rather than foliar in nature, bearing both leaves and sporangia, such a sporophyll does not seem to me to be comparable with the fertile branch of *Tmesipteris*.

Lignier[6] has placed the Psilotaceae in a position which implies their near relationship to the 'Protopteridophyta', and supposes that they arose from that stock before the first Protofilices, which class gave rise to the Filices, Equisetales, and Sphenophyllales, were evolved. He lays stress on a connexion between the Psilotaceae and the Lycopodiaceae.

On the whole the relationship of the Psilotaceae must be acknowledged to be still obscure, and for the present it seems best to place them in a separate cohort, the Psilotales, as has been suggested by Scott and others.

[1] Baker, 1887, p. 29. [2] Campbell, Mosses and Ferns, 1905, p. 485.
[3] Scott, Studies, 1900. [4] Thomas, 1902. [5] Bower, 1903. [6] Lignier, 1903, p. 95.

VII. Summary.

In conclusion it may be well to give a short summary of the principal facts demonstrated in the course of the above research.

An endodermis has been shown to be present in the rhizome surrounding the single stele and has characteristic markings on its radial walls. At the transition region the endodermis does not alter its position, but it becomes less and less obvious and loses these characteristic markings; in the aerial stem it can no longer be distinguished as a definite layer. If we are to look upon the endodermis as of morphological significance, being the layer separating cortex from stele, it becomes impossible to consider the pith, which arises in the centre of the stele in the transition region and quickly expands to form a large tissue in the stem, of other than stelar origin. While in no way wishing to lay stress on such a view of the endodermis, it is interesting to note that here we have a protostele passing into a medullated monostele without the intermediate stage of solenostele, and this medullated monostele is already present at a level at which no leaves have yet arisen.

The statements of other authors concerning the growth of the plant from a single apical cell have been confirmed, both in the rhizome and stem apex.

The absence of sterile branches is a curious feature in the plants supplied to me. It must be sufficient to notice here that the course of the vascular bundles in a sterile branch is described by Bertrand, and is exactly similar to that found in a fertile branch; the single bundle entering the axis branches into three, the two lateral traces supplying the leaves, and the central one presumably representing the vascular supply of the apex.

In the fertile branches the central of the three bundles supplies the synangium which occurs at the point of divergence of the two leaves. A single trace thus enters the synangium pedicel and then branches again into three, the central one soon terminating, while the two lateral ones diverge, and run round the periphery of the septum. The central trace, described now for the first time, seems an important piece of evidence in favour of the axial theory of the sporophyll in the Psilotales, and is here regarded as representing the vascular supply of the apex of the branch.

This theory appears to me to be also supported by the abnormalities described both by Professor Thomas and in this paper. It is concluded that the fertile branch in *Tmesipteris* is of cauline nature, and consists of an axis, bearing two leaves or a single dichotomously branched leaf (which have possibly only become associated with it in the course of evolution), and terminating just above their origin. At its apex is borne a synangium which is formed from one or two masses of sporogenous tissue, which have fused over the apex of the branch.

The extraordinary resemblance between such a form as *Sphenophyllum majus*, with its lax cone and simple sporophylls, and *Tmesipteris*, causes one to search for some evidence of phylogenetic relationship. In this connexion the theory that the sporophyll in the Psilotales is a lobed leaf has received much attention. The question is here raised as to whether it is possible to look upon the sporophylls in the Sphenophyllums as organs in which the branch axis has become much reduced or is wanting, each so-called 'sporophyll' being morphologically equivalent to a branch bearing leaves and sporangia.

The similarity found to exist in the methods of formation of leaf and branch traces in *Tmesipteris* is of some interest in a member of Jeffrey's class Lycopsida. The difference is shown to be one of degree only, the presence of a gap depending on the greater length of time elapsing between the division of a xylem group to form a trace and the departure of that trace from the stele, during which time the trace has assumed the appearance of a separate constituent of the stem stele. Both leaf and branch traces are originally formed in exactly the same way, by the division of a xylem group of the stem, half the protoxylem and all the centrifugal xylem of which becomes surrounded by phloem and give rise to the trace. Such facts appear to militate against Jeffrey's hypothesis of the essential importance of gaps.

Finally, it is concluded that the relationship of the Psilotaceae are not sufficiently evident as yet to warrant a position especially near any family, and that they are better retained alone in the cohort of Psilotales.

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EXPLANATION OF PLATES VII AND VIII.

Illustrating Miss Sykes' paper on *Tmesipteris*.

The figures were drawn with the aid of Swift's ⅔ and ⅛ lenses, No. 3 ocular, and a Zeiss-Abbé camera-lucida.

Fig. 1. Transverse section of the rhizome near the apex; *a* = cortical cell containing starch; *b* = cortical cells filled with fungus hyphae; *c* = rhizoid. × 116.

Fig. 1 *a* is a single cell of the endodermis of the rhizome (more highly magnified than in Fig. 1), showing starch contents and the thickenings on its radial walls. Fig. 1 *b* is a single cortical cell of the rhizome, magnified as Fig. 1 *a*, and showing a large number of fungus hyphae. × 400.

Fig. 2. Transverse section taken from the base of a large aerial stem; *a* = innermost layer of cortical cells, containing a brown substance; m^1 = base of very small scale-leaf in transverse section; m^2 = larger scale-leaf with stomata and assimilating tissue, but no vascular supply; at a^1 one of a few cortical cells opposite this scale-leaf is seen to be invaded by the brown substance which surrounds the stem stele; m^3 = group of stem stele in which the protoxylem is in the act of dividing to give rise to the first leaf-trace. × 78.

Fig. 3. First leaf-trace in its passage through the cortex. This is the leaf-trace seen arising in Fig. 2, m^3, and supplies the last scale-leaf. × 400.

Fig. 4 *a*, *b*, *c*, are successive sections of a series taken near the apex of an aerial stem. They show three stages in the formation of a branch stele. × 116.

Fig. 5. Transverse section of half of a leaf of *Tmesipteris tannensis*. × 78.

Fig. 6. Surface view of small piece of epidermis from a leaf of *T. tannensis*, showing the thickening bands on the walls of the epidermal cells. × 400.

Fig. 7. Transverse section taken half way up a fertile branch (cortex somewhat diagrammatic). At *a* and *b* are the decurrent bases of the two leaves, each with their two groups of stomata. × 78.

Fig. 7 *b* shows some sieve tubes in longitudinal section, drawn from a section of a fertile branch; the darkly shaded portions are stained green, while the dotted areas or sieve plates are pink. × 400.

Fig. 8. Transverse section of fertile branch at the origin of the synangium, passing longitudinally through the pedicel of the synangium; *a* and *b* = traces of the two leaves; the bundle cut longitudinally is that supplying the synangium and contains three groups of tracheides surrounded by phloem (diagrammatic); *S* = tissue of septum. × 78.

Fig. 9. Transverse section through synangium pedicel, taken at right angles to Fig. 8, at level I showing a single trace. The small-celled tissue is the tissue of the pedicel, while the larger cells surrounding it represent the cortex of the branch. × 87.

Fig. 10. As Fig. 9, but from a section taken at level II in Fig. 8. The single trace has now branched into three. × 116.

Fig. 11. From the same series as Figs. 9 and 10, but at level III in Fig. 8. The middle trace has disappeared, and the two lateral ones have diverged to either side of the synangial septum. This section passes through the middle of the synangium, in a direction tangential to its pedicel. × 87.

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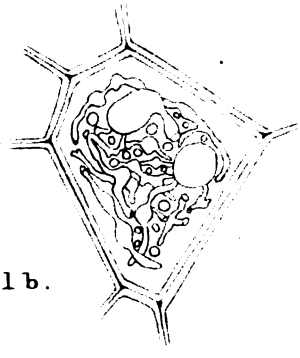

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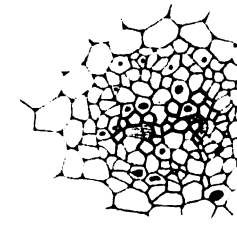

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SYKES — TMESIPTERIS.

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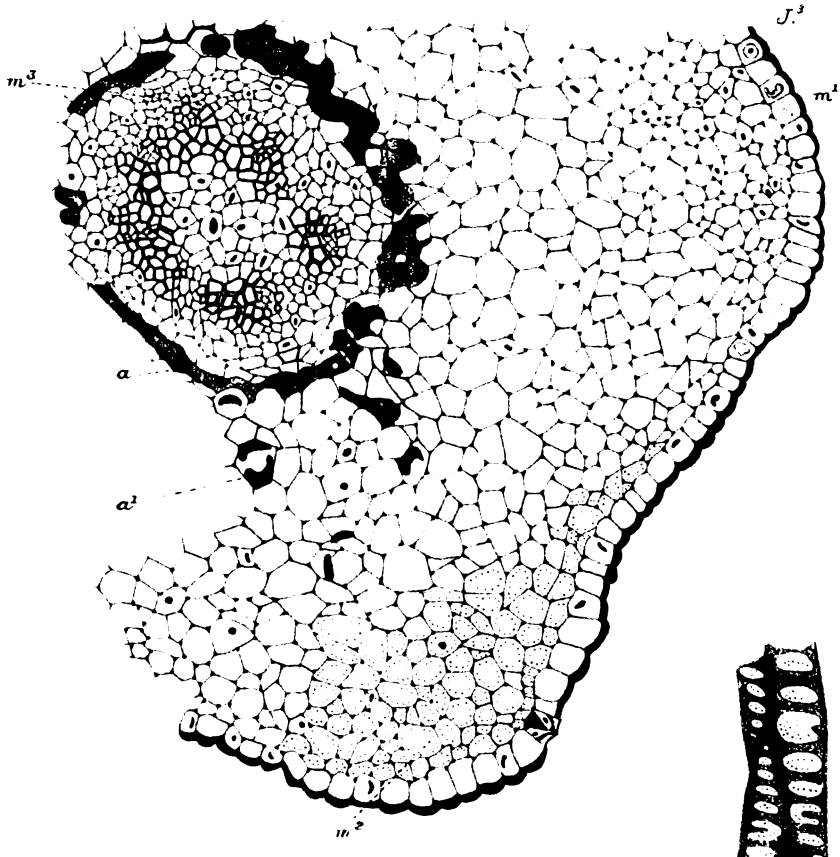

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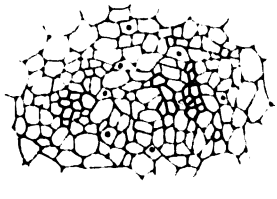

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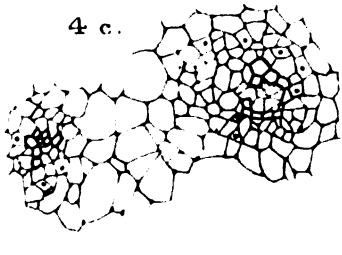

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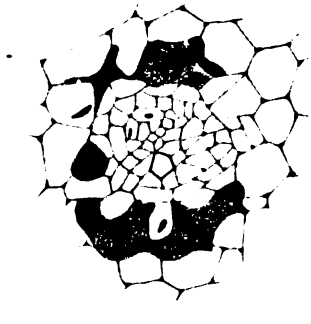

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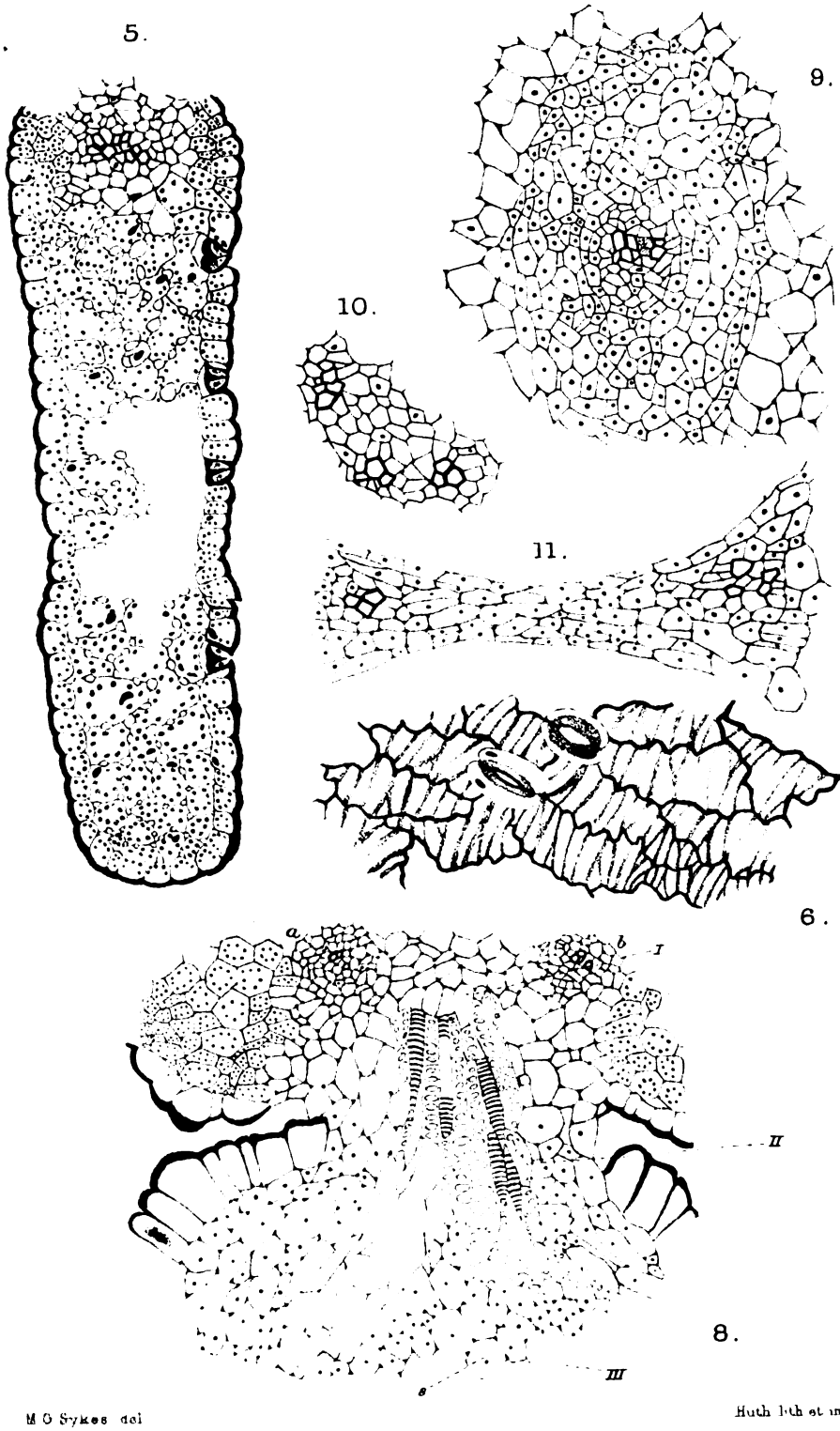

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SYKES — TMESIPTERIS.

Studies on some Javanese Anthocerotaceae. II.

BY

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Professor of Botany in Stanford University.

With Plates IX and X, and two Figures in the Text.

THE conditions in Western Java are especially favourable for the growth of Hepaticae, and among these is a large number of species of the Anthocerotaceae, of which many were collected. In a recent paper (Studies on some Javanese Anthocerotaceae, I, Ann. of Bot., vol. xxi, p. 467) two of these species, which were referred to a new genus, *Megaceros*, were described. The present paper is concerned with the species of *Dendroceros* and *Notothylas*, collected at the same time.

The species of *Dendroceros* are all tropical, and the first account of the development of these we owe to the researches of Leitgeb.[1] Leitgeb studied several species of *Dendroceros*, and although he had only herbarium material to work with, he succeeded in making out the most important points of structure in the reproductive organs and embryo. The writer collected in Jamaica two species, *D. Breutelii*, N. ab E., and *D. crispus*?, and was able to add a number of details which the insufficiency of his material prevented Leitgeb from noting.[2]

But one species of *Dendroceros*, *D. javanicus*, N. ab E., has been hitherto recorded from Java,[3] but during the writer's stay in the island two very distinct species were collected, one at Tjibodas, the other at Lebak Saat on Mount Gedeh. Search was made for *Dendroceros* in the neighbourhood of Buitenzorg, but without success. As the species collected at Tjibodas was very inconspicuous and difficult to find, it is quite likely that the same or some similar species may occur at the lower level.

The original description of *D. javanicus* was not available, and for the present it must remain uncertain which (if either) of the species here described is the true *D. javanicus*. At Tjibodas, on the trunks of two or

[1] Untersuchungen über die Lebermoose, iv, 1879.

[2] Campbell, On the Structure and Development of *Dendroceros*, Nees, Journ. Lin. Soc. London, xxxiii, p. 467, 1898.

[3] Schiffner, Conspectus Hepaticarum Archipelagi Indici, Batavia, 1898.

[Annals of Botany, Vol. XXII. No. LXXXV. January, 1908.]

three trees near the edge of the forest, there was found growing a small species (Pl. IX, Fig. 4), which was assumed to be *D. javanicus*. Although careful search was made in various localities, no other specimens were found. A second, much larger species (Fig. 1) was subsequently met with nearer the summit of Mount Gedeh. This was only found once, but was growing abundantly on the twigs of an undetermined shrub, and was quite conspicuous, while the other species growing among the mosses and liverworts on the bark of trees was very hard to find. The large species was growing at an elevation of 2,200 metres, which was rather unexpected, as *Dendroceros* usually prefers the warmer regions nearer sea-level.

The determination of the two species must be left until a comparison can be made with authentic specimens of *D. javanicus*. We shall simply refer to them here as species A, the large form from the higher station, and species B, the smaller form from Tjibodas. The former species is very much larger than the other (see Pl. IX, Figs. 1 and 4), and in many respects seems to agree with the plant which Leitgeb studied under the name *D. javanicus*.[1] The magnification of Leitgeb's figures is not indicated, and it is impossible therefore to judge of the size of the plant studied by him. The thallus of form A (Fig. 1) is about 3 cm. in length, and more or less regularly pinnately branched. The midrib, as in all species of *Dendroceros*, is sharply defined, and the wings of the thallus consist of a thin lamina composed of but a single layer of cells. This lamina in the species in question is very much lobed and folded, so that it closely resembles such a liverwort as *Fossombronia*, the lobes of the lamina simulating leaves. In this respect it resembles Leitgeb's figure of *D. javanicus*, but the margin is even more irregular than in his figure. It also resembles the West Indian *D. crispus*, but is very much larger than any specimens of that species collected by the writer in Jamaica, and the margin of the thallus is very much more lobed and folded. The cells of the lamina are destitute of the large lacunae which are very conspicuous in the Tjibodas plant (Fig. 6), and in this respect it differs from Leitgeb's plant, which is described as having large lacunae in the lamina. The cells of the lamina show the collenchyma-like thickenings at the angles that Leitgeb describes in *Dendroceros Breutelii*. The midrib is very massive, and contains large intercellular spaces, but these are not air-chambers, as Leitgeb states. He compares them to the air-chamber of the Marchantiaceae, but they are filled with mucilage, as they are in other Anthocerotaceae where such spaces are present. Leitgeb was probably misled by having to deal with dried material, where the drying up of the mucilage or its colourless character may have made him overlook the contents of these spaces. In stained microtome sections it is very evident that these intercellular spaces are not air-chambers.

[1] Leitgeb, loc. cit., Plate II, Fig. 20.

The antheridia of this species, which are very large, occur upon short lateral branches (Fig. 2 δ), and in this respect also the plant resembles Leitgeb's figures and description of *D. javanicus.*

The sporogonium is not very long, being only about 1 cm. in length, but is relatively stout, and opens along one suture (Fig. 3). The delicate columella may be seen projecting from the opening. The involucre is about half the length of the sporogonium.

The second species was found at Tjibodas, but only in a very limited area. As already stated, it was growing among mosses and liverworts upon the trunks of a few trees near the edge of the forest adjoining the garden. Although repeated search was made at other places, no more specimens were found. It is an excessively delicate little plant, and hard to recognize except when in fruit. It is only about one-third the size of the other species, and the thallus is dichotomously branched (Fig. 4). The wings of the thallus are very little folded or lobed, and the plant looks almost like a *Metzgeria*, and is not much larger than the common species of that genus. The wings contain very conspicuous lacunae (Fig. 6), in which respect it differs strikingly from the species A, but agrees with Leitgeb's account of *D. javanicus.* There are no lacunae in the midrib.

The antheridia and archegonia are both upon the same shoots, and the antheridia are much smaller than in the species A. The sporogonia are very slender, but are longer than in species A. The involucre is long, and the dehiscence is along one suture as in the other species (Fig. 5).

The Anatomy of the Thallus.

The form of the apical cell and its segmentation agree entirely with those of the other species that have already been investigated. The form of the apical cell (see Figs. 8 and 9) is very much like that of *Pellia epiphylla* and that of the prothallium of most Ferns, and it differs from that of the other genera of Anthocerotaceae in its forming basal segments extending the whole depth of the thallus. The two species are alike in the form and segmentation of the apical cell, but the species A has a thicker midrib and develops the conspicuous intercellular species already referred to (Fig. 9, *l*). These spaces are quite absent from the species B.

As in most Anthocerotaceae, each cell of the thallus contains a single large chromatophore, in which is a very distinct pyrenoid (Fig. 7, *p*). The nucleus, *n*, is small and not at all conspicuous, but it is readily demonstrated.

The Reproductive Organs.

The archegonia (Figs. 9 to 12) are not in any way different from those of the other species that have been examined, and closely resemble those of *Anthoceros.* The neck-cells are usually slightly broader than in

Anthoceros, but this is not very marked. As in other Anthocerotaceae, the archegonium projects but slightly above the level of the thallus. There are from two to four cover-cells in the ripe archegonium (Fig. 13), and these are thrown off when the archegonium opens. The neck-canal-cells usually number five.

The antheridia (Figs. 14 to 22), as in other species of *Dendroceros*, are borne singly in the antheridial cavity. No attempt was made to follow out the early development, as there was no indication that this differed in any marked degree from that of the other species that had been studied. There is no doubt of the endogenous origin of the antheridium, and, as in all other described species of *Dendroceros*, the archegonium lies nearly horizontal in the antheridial chamber and develops an extremely long pedicel, which is coiled up in the cavity. Leitgeb supposes that at maturity the stalk straightens out and projects the archegonium above the surface of the thallus. This point, however, was not investigated. The body of the antheridium is oval, and very early in its history the single layer of peripheral cells is separated from the inner spermatogenic tissue. Cross-sections of the pedicel (Fig. 19, *p*) show it to be composed of two rows of cells in all the specimens examined in both species. This agrees with Leitgeb's account of the species studied by him, but in *D. Breutelii* there may be four rows in some cases.[1]

The spermatozoids are too small to make a study of the spermatogenesis satisfactory, and no attempt was made to trace the development of the spermatozoids. After the last mitosis the spermatocytes are in pairs, apparently not separated by a cellulose membrane (Fig. 18). This is much like what occurs in many Hepaticae. Presumably the cilia arise from a blepharoplast, but this point was not demonstrated. The mature antheridium is very large in the species A (Fig. 19), being about 350 μ in diameter. This is more than twice the size Leitgeb gives for *D. javanicus* (loc. cit., p. 32). The antheridium in the second species is much smaller (Fig. 22).

The Embryo.

Leitgeb describes the older embryos of *Dendroceros*, but did not obtain the earlier stages. The writer succeeded in obtaining younger stages of *D. Breutelii* (loc. cit.), but the first divisions were not seen, although it was conjectured that the first division was a longitudinal one, as in *Anthoceros*. This has proved to be also the case in the two species under consideration (Fig. 23), and it will be safe to assume that this is the rule in *Dendroceros*. The second walls are transverse and nearly median. This first transverse division probably determines the boundary between the foot and the capsule. The next divisions are vertical, but

[1] Campbell, loc. cit., p. 472.

to judge from transverse sections of the young embryo they are somewhat variable in position. Fig. 25 shows three sections of a young embryo of six cells. In this case the upper region was divided into four cells, but they were not quite regularly disposed. The foot-region consisted at this stage of but two cells. Fig. 24 shows a nearly median longitudinal section of an 8-celled embryo of the larger species, A, and in this case also the octant walls do not all fall in the same plane. The foot-cells have already begun to grow out into the root-like extensions, which later are so conspicuous.

The differentiation of the columella (endothecium) is the same as in *Anthoceros* (Fig. 26), and the separation from the amphithecium of a single layer of archesporial tissue is also the same as in *Anthoceros*, except that, as was pointed out in *D. Breutelii* and *D. crispus* (Campbell, loc. cit.), the archesporial layer extends quite to the transverse wall that marks the upper limit of the foot. In this respect *Dendroceros* closely resembles *Megaceros*, which in some other respects is also more like *Dendroceros* than it is like *Anthoceros*. There are, moreover, further periclinal divisions in the apical part of the sporogenous layer which otherwise remains but one cell thick (Figs. 27, 28). This is, however, less marked than in *Megaceros* or *Notothylas* (see Text-figs. 1 and 2).

The columella in the two Javanese species was better developed than in those from the West Indies, this being especially the case in species A. In cross section (Fig. 29), instead of the sixteen cells usually found in *D. Breutelii* there were sometimes nearly twice as many. Leitgeb figures a similar large columella in *D. cichoraceus*.

As in *D. Breutelii* and the other species studied by Leitgeb, the sporogenous layer, except above the apex of the columella, remains but one cell in thickness or very imperfectly duplicated in places. This is much like the smaller species of *Anthoceros* such as *A. laevis*.[1] In the larger species, e.g. *A. Pearsoni* (Text-fig. 2, C), the doubling of the sporogenous layer is complete. In *Megaceros*, which in so many ways closely resembles *Dendroceros*, there are from three to four layers of cells when the cell-divisions are completed (Text-fig. 2, B).

Fig. 29 shows a transverse section of the sporogonium of species A some distance above its base. The large columella is surrounded by a single layer of archesporial cells, while the outer part of the sporophyte consists of from four to five layers of cells, which develop chlorophyll. The number of layers of cells in the wall may, in the larger species, become seven or eight. In no cases were stomata observed, and this agrees with all the other species of *Dendroceros* as well as with *Megaceros*.

No rule could be made out as to the origin of the fertile and sterile cells of the archesporium. The spore-mother-cells can soon be

[1] See Davis, the Spore Mother Cell of *Anthoceros*, Bot. Gaz., xxviii, 1899.

recognized by their rounded form and greater diameter. The sterile cells remain narrow and are soon easily distinguishable from the rounded spore-mother-cells. The division of the latter was not followed, as there was no evidence that it was different from the other forms that have been studied. The two Javanese species do not differ much in the size of the ripe spores (Fig. 30), but the species A has both the columella and the outer tissue much more developed than the smaller one. In the latter there are seldom more than four layers of cells in the sporogonium-wall, while in the larger species there may be as many as eight. The superficial cells of the smaller species are longer and narrower than in the larger one, and in the latter they often project slightly.

After the division of the spore-mother-cell is completed, the spores remain together in tetrads until they are almost ready to be discharged from the sporogonium. While still together, a thin, finely papillate perinium is developed and the spores undergo repeated division, so that each one is a multicellular body of considerable size (Fig. 30). Both of the Javanese species agree in this respect, and this is known to be the case also in a number of others, e.g. *D. crispus*, *D. cichoraceus*. The cell-mass derived from the spores has a large amount of chlorophyll, and really germination begins inside the sporogonium, as it does in *Pellia* and *Fegatella*.

The elaters, as in the other species of *Dendroceros*, are multicellular, and are to be considered as fragments of the net of sterile cells which incloses the spores. There is a broad spiral band of a yellowish brown colour, which is scarcely interrupted by the division walls between the cells of the elater (Fig. 31).

Notothylas javanicus.

In the garden at Buitenzorg, and at other places in the vicinity, a species of *Notothylas*, presumably *N. javanicus*, was not at all uncommon. Material of this species was collected in order to compare it with the common American species, *N. orbicularis*, which is the only species that has been completely investigated.[1] The statement of Leitgeb, that the columella in *Notothylas* is not infrequently quite absent or may be of secondary origin, was not confirmed by Mottier in the case of *N. orbicularis*. *N. javanicus* was examined carefully with reference to the same point, and the conclusions reached agree essentially with those of Mottier with reference to *N. orbicularis*. *N. javanicus* closely resembles in general appearance *N. orbicularis*. The thallus is usually nearly orbicular, due to a repeated and rapid dichotomy. It is somewhat yellowish-green in colour, and the sporogonia project but little from the involucre. Where

[1] Mottier, Contributions to the Life History of *Notothylas*, Ann. of Bot., viii, 1894; Campbell, Development of the Mosses and Ferns, First Ed., 1895.

the sporogonia are abundant the short, nearly horizontal capsules occur quite thickly set round the margin of the thallus. Quite often dwarf sporogonia are met with, resembling those described in other species, and like these are probably due to imperfect nutrition.

Sections of the thallus of *N. javanicus* show it to be quite solid, and no trace of the mucilage-cavities which are conspicuous in *N. orbicularis* can be found. The apical growth (Pl. X, Figs. 32, 33) is exactly like that of *Anthoceros* and *Megaceros*, there being two sets of segments, dorsal and ventral, cut off from the large initial cells.

The Reproductive Organs.

The archegonia are similar in origin and development to those of *Anthoceros*, but they are somewhat broader in the early stages, and the number of neck-canal-cells is usually less. In all the specimens of *N. javanicus* that were examined there were but three neck-canal-cells (Figs. 32, 34), and Mottier found this to be true in *N. orbicularis*, although the writer found as many as five in the latter species. The number of ripe archegonia that were examined in *N. javanicus* was not very large, and it is not impossible that in this species also there may exceptionally be more than three neck-canal-cells.

In some cases the neck-cells are cut off by a longitudinal wall after the first transverse cell of the young archegonium has been formed (Pl. X, Fig. 33). In such cases the neck-canal-cells are narrower than when this longitudinal wall is suppressed. An abnormal case was seen in which the egg-cell had divided into two by a longitudinal division wall after the cutting off of the ventral canal-cell (Fig. 34). There are usually two cap-cells present. Whether more than this may be formed in *N. javanicus* was not determined. The antheridia (Fig. 35) are formed usually four together in the antheridial cavity, and apparently do not differ in any important particular from those of *N. orbicularis*.[1]

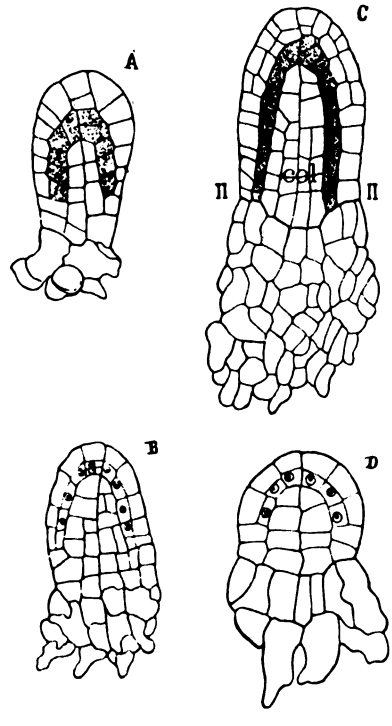
The Embryo.

Neither Leitgeb nor Mottier described the earliest stages of the embryo. The latter thinks that in *N. orbicularis* the first division-wall is transverse. This does not seem to be the case in *N. javanicus* to judge from the few young embryos that were examined. In one case a two-celled embryo was sectioned (Fig. 36). This was divided longitudinally exactly as in *Anthoceros*. The next youngest embryo seen (Fig. 37) was divided into three tiers by two transverse sets of walls, and in this case at least it was clear that the development of the columella and archesporium was confined to the uppermost segment of the embryo

[1] See Campbell, Mosses and Ferns, Second Ed., Fig. 80.

as in *Anthoceros*. The small endothecium in this case was cut off by a periclinal wall. Fig. 38 shows a median longitudinal section of an older embryo in which the archesporium is completely differentiated. It agrees entirely with *N. orbicularis* in this respect; and as Mottier showed, the archesporium is of amphithecial origin and formed in exactly the same way as in *Anthoceros*.

Cross-sections of embryos of about the same stage as that represented in Fig. 36, show much the same appearance as similar sections in the other genera, except that the columella is much less developed. While in

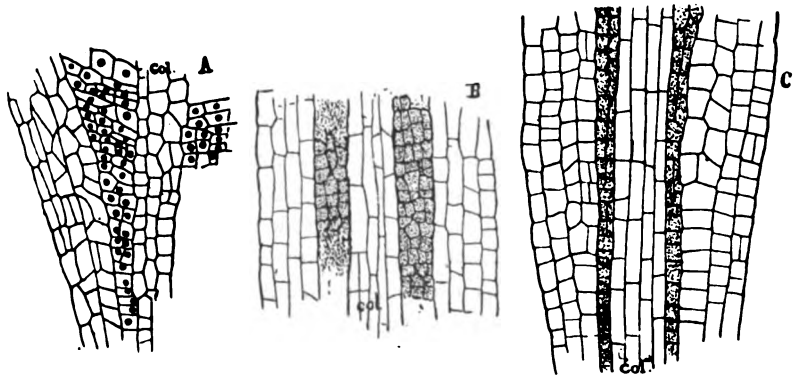


TEXT-FIG. 1. Median longitudinal sections of embryos of about the same age, showing the extent of the archesporium in the four genera of Anthocerotaceae. A. *Notothylas orbicularis*; B. *Dendroceros Breutelii*; C. *Megaceros tjibodensis*; D. *Anthoceros Pearsoni*.

the larger sporogonia (Fig. 44) the columella may show three cells in longitudinal section, most of the smaller ones showed but four cells in cross-section, and it is less developed than in *N. orbicularis*. In some specimens but three cells were seen in cross-section (Fig. 42). This would indicate that the columella-cells were entirely suppressed in one of the quadrants of the embryo, and presumably in this quadrant the endothecium would contribute to the sporogenous tissue. In no specimens that were examined, however, was the columella entirely absent, and

it is doubtful whether this is ever the case, as Leitgeb supposed it to be. Except in microtome sections it would be quite impossible to recognize the slender columella obscured by the massive sporogenous tissue which surrounds it. The sporogonium becomes pear-shaped (Fig. 43), and the single layer of sporogenous tissue increases very much in thickness, becoming ultimately three or four cells thick (Fig. 44). Above the summit of the columella, which is not always very clearly defined in the upper region, the sporogenous tissue divides rapidly and forms a large mass (Fig. 43). The differentiation of the sporogenous and sterile cells of the archesporium is entirely similar to that of *N. orbicularis*. There is a pretty regular alternation of horizontal layers of fertile and sterile cells, the latter (*sp*) very soon becoming recognizable on account of their larger size.

The foot in *Notothylas* is much smaller than in *Dendroceros* and



TEXT-FIG. 2. Median sections near the base of older sporophytes of *Notothylas* (A), *Megaceros* (B), and *Anthoceros* (C), showing the relative size of the archesporium in the three genera.

Anthoceros, corresponding to the smaller size of the sporophyte. The root-like outgrowths are, however, well developed and appear at an early stage in the growth of the embryo (see Figs. 38, 39).

It is clear that in normal cases, at least, the sporogonium of *N. javanicus* develops in precisely the same way as that of *N. orbicularis* or the other Anthocerotaceae. There is no evidence of a secondary formation of the columella from potentially sporogenous tissue, as Leitgeb surmises is the case in some forms of *N. fertilis*. The great reduction of the columella in some of the stunted sporogonia makes it quite conceivable that this may be carried a stage further and that sporogonia might be formed without a columella; but no cases of this were seen, although a good many young sporogonia were sectioned, and there is no question that usually, at least, a columella is formed and that it always originates in the same way as that of the other Anthocerotaceae.

Affinities of the Anthocerotaceae.

Notothylas is without doubt the simplest and probably the most primitive of the Anthocerotaceae. Of the other genera *Anthoceros* closely resembles *Notothylas* in the general character of the thallus and sexual organs. *Megaceros* is to some extent intermediate between *Anthoceros* and *Dendroceros*, resembling the former in the general structure of the thallus, but having the solitary antheridium characteristic of *Dendroceros*. Whether the single antheridium is a more primitive character than the group of antheridia found in *Notothylas* and most species of *Anthoceros* it is impossible to determine. The question is also open as to whether the type of thallus in *Notothylas* and *Dendroceros* is the more primitive. A comparison might be made with the type of thallus found in *Pallavicinia* and *Aneura* among the Jungermanniaceae. In regard to the sporophyte, *Notothylas*, both in the smaller size and in the relatively larger amount of sporogenous tissue, is clearly the lowest member of the family. Of the other genera, *Megaceros* most nearly resembles *Notothylas* in the extent of the sporogenous tissue (Text-Fig. 2, B), but the columella and outer sterile tissue are very much better developed, and the sporogonium may reach a very large size, rivalling the largest species of *Anthoceros*, which, on the whole, it perhaps most nearly resembles, although the spiral elaters and absence of stomata are more like *Dendroceros*. In *Dendroceros* and the smaller species of *Anthoceros*, e.g. *A. laevis*, the sporogenous tissue is reduced to a single layer except at the summit, where in *Dendroceros* (Text-Fig. 1, C) there may be a considerable increase in the amount of fertile tissue. The larger species of *Anthoceros*, with their highly developed assimilative tissue and perfect stomata, may probably be considered as the highest existing form of this peculiar form of sporophyte.

The next question to be considered is the relation of the Anthocerotaceae to other forms. The peculiar solitary chromatophore characteristic of most of these is so closely similar to that of many green Algae that it is probable it is an inheritance from some algal ancestor. This peculiarity has been supposed to be universal among the Anthocerotaceae, but it has been recently shown that in the group of the genus *Anthoceros*, which the writer has proposed to separate as a special genus, *Megaceros*, there are normally several chromatophores in the inner cells of the thallus, and that these chromatophores are usually destitute of a pyrenoid and in all respects closely resemble the normal chromatophores of the higher plants.

Leitgeb has assumed a remote relationship of *Notothylas* with the Jungermanniales, and believed that he had found sporogonia of the former in which the sporogenous tissue arose from the endothecial tissue.

As we have pointed out, this formation of the sporogenous tissue from the central part of the sporogonium has not been confirmed by the writer's study of *N. javanicus*, although the slight development of the columella in some of the small sporogonia indicates the possibility of a complete suppression of the columella in the poorly nourished sporogonia.

A comparison of the sporogonium of *Notothylas* is perhaps best made with *Sphaerocarpus* or even better with *Cyathodium*.[1] The latter is especially interesting, as the foot of the sporogonium, unlike that of any other liverwort, produces root-like extensions like those of the typical Anthocerotaceae. It is also worthy of note that the Targionieae, and especially *Cyathodium*, are characterized by remarkably large chromatophores, those in *C. foetidissimum* being sometimes reduced to four in some of the assimilative cells. The writer has examined this point in specimens collected in Java, and the resemblance to the chromatophores of *Megaceros* is sufficiently striking. Should these resemblances in *Cyathodium* and the Anthocerotaceae prove to be anything more than coincidences it would show that the affinities of the Anthocerotaceae are rather with the Marchantiales than with the Jungermaniales. The relationship in either case is certainly remote, and for the present at least it will probably be best to regard the Anthocerotaceae as sufficiently distinct from the true Hepaticae to form a special class, Anthocerotes, as was suggested by Howe.[2]

EXPLANATION OF PLATES IX AND X.

Illustrating Professor Campbell's Studies on Anthocerotaceae, II.

PLATE IX.

Fig. 1. *Dendroceros sp.* (A). Thallus. × 3.
Fig. 2. Apex of a shoot with antheridial branch, ♂. × 3. *sp*, young sporophytes.
Fig. 3. Two ripe sporogonia of the same. × 3. *col*, columella; *in*, involucre.
Fig. 4. Plant of *Dendroceros sp.* (B) with two sporogonia, *sp*. × 3.
Fig. 5. A ripe sporogonium of the same. × 3.
Fig. 6. Cells of the wings of the thallus of B, showing the large lacunae. × 110.
Fig. 7. A single cell showing the chromatophore with the included pyrenoid, *p*. × 280. *n*, nucleus.
Fig. 8. Apical region of the thallus of B, longitudinal section. × 280.
Fig. 9. A similar section of A; *ρ*, archegonia; *l*, mucilage cavities.
Fig. 10. Young archegonium of A. × 480.
Fig. 11. Young archegonium of B. × 480.
Fig. 12. Open archegonium of B. × 280.

[1] Lang, On the Morphology of *Cyathodium*, Ann. of Bot., xix, p. 411, 1905.
[2] Mem. Torrey Botanical Club, vii, 1899.

Fig. 13. Transverse section of the apex of the archegonium, showing three cover-cells, *d*. × 280.

Fig. 14. Longitudinal section of an antheridial shoot of A. × 25. *n*, *Nostoc* colony; *M*, mucilage cavities; ♂, antheridia.

Figs. 15–17. Development of the antheridium in A. × 280.

Fig. 18. Spermatogenic cells after the last division. × about 600.

Fig. 19. Ripe antheridium of A. × 110. *p*, section of the pedicel.

Fig. 20. Young antheridium of B. × 280.

Fig. 21. An older antheridium of B. × 280.

Fig. 22. Ripe antheridium of B. × 110.

Fig. 23. Two longitudinal sections of a two-celled embryo of A. × 280.

Fig. 24. An 8-celled embryo of A. × 280.

Fig. 25. Three transverse sections of a 6-celled embryo of B. × about 600. *a*, apex; *c*, base.

Fig. 26. Two longitudinal sections of a young embryo of B. × 280. The separation of endothecium and amphithecium is complete.

Fig. 27. Median longitudinal section of an older embryo of A. × 280. The archesporial tissue has the nuclei shown.

Fig. 28. Longitudinal section of the young sporophyte of A, showing the extent of the archesporial tissue, *sp*; the columella, *col*; *f*, the foot. × 65.

Fig. 29. Transverse section near the base of the young sporophyte of A; the archesporial cells have the nuclei shown. × 280.

Fig. 30. Section of ripe spore tetrad of A, showing the cell-divisions in the spores. × 280.

Fig. 31. Fragment of a ripe elater. × 280.

PLATE X.

All figures refer to *Notothylas javanicus*, N. ab E.

Fig. 32. Longitudinal section of the apex of the thallus with a nearly mature archegonium, ♀; *x*, apical cell. × 280.

Fig. 33. A similar section, with young archegonium. × 600.

Fig. 34. An abnormal archegonium with the egg-cell divided into two. × 480. *v*, ventral canal-cell.

Fig. 35. Nearly mature antheridium. × 280.

Fig. 36. Two-celled embryo. × 480.

Fig. 37. An older embryo. × 480. *II*, first transverse wall; *en*, one of the primary endothecium-cells.

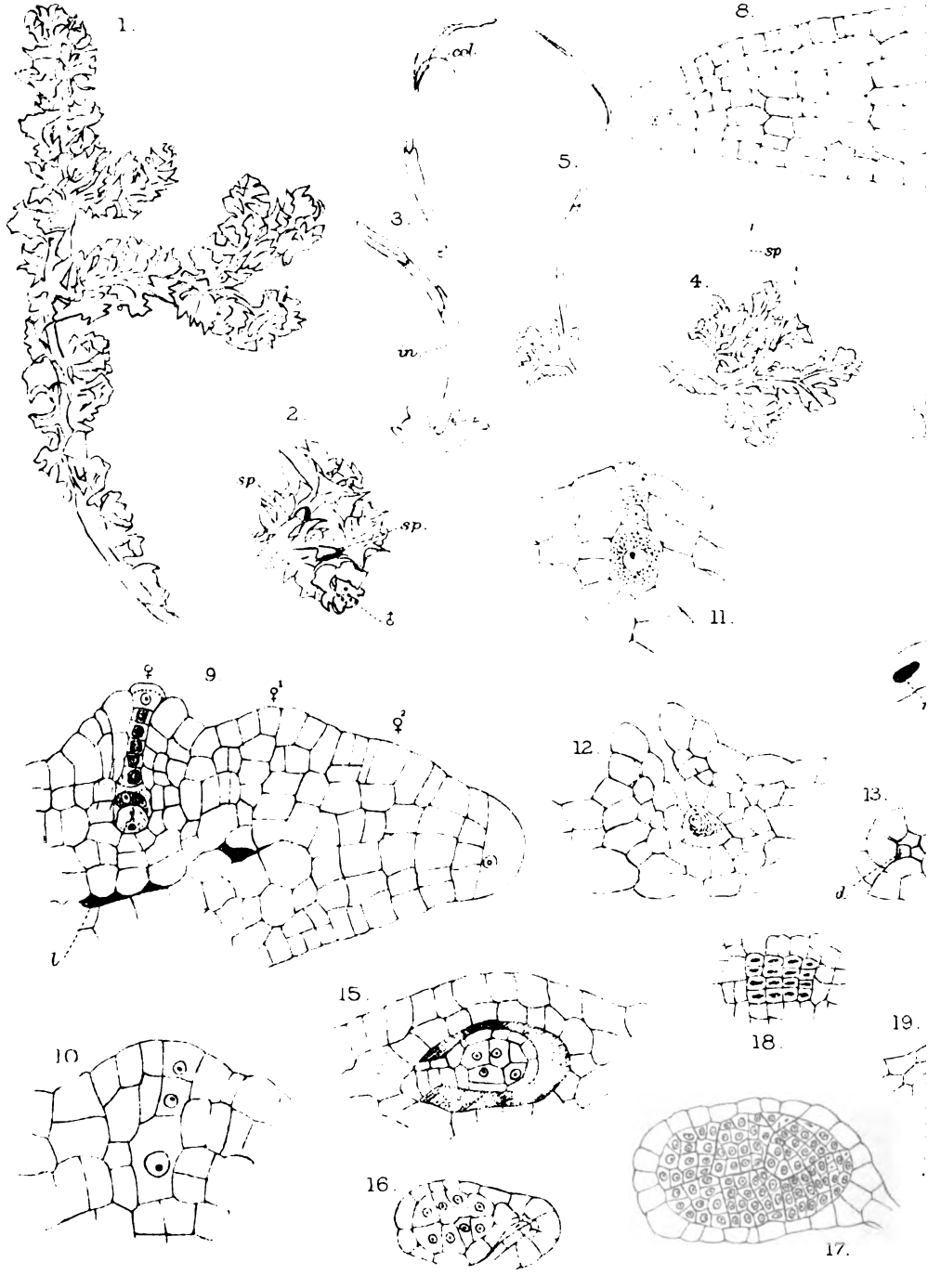
Fig. 38. Median longitudinal section of an older embryo; the archesporial cells show the nuclei. × 280.

Figs. 39–41. Five transverse sections of an older embryo. × 280. *a*, *b*, through the foot; *c*, *d*, nearly median sections; *c*, above the summit of the columella.

Fig. 42. Transverse section of an older embryo, showing but three rows of cells in the columella. × 280.

Fig. 43. Median longitudinal section of young sporophyte. × 280. The archesporium is shaded.

Fig. 44. Median longitudinal section near the base of a large sporophyte; *col*, columella; *sp*, fertile; *el*, sterile cells of the archesporium. × 280.



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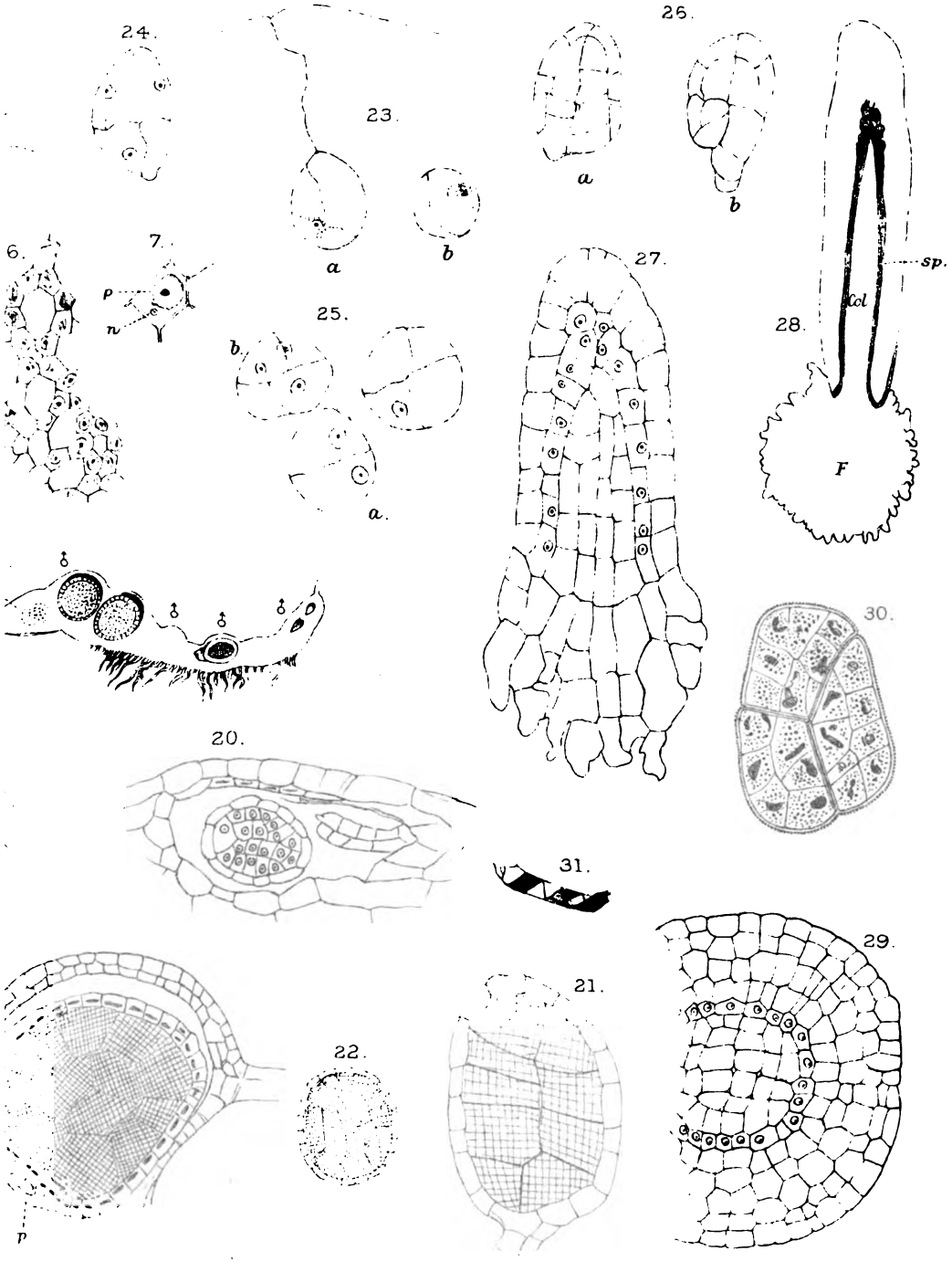
CAMPBELL— ANTHOCEROTACEAE.



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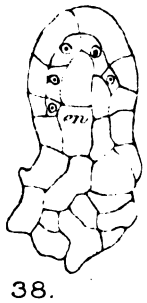

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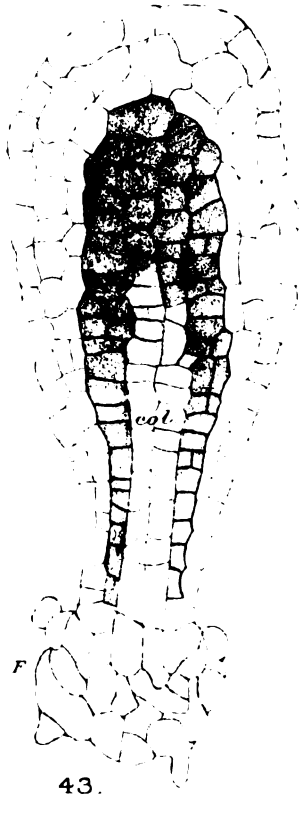

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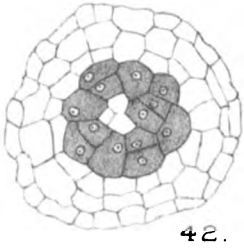

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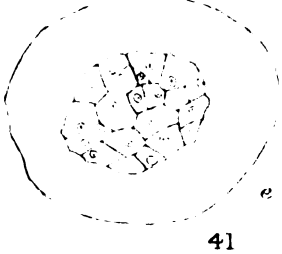

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44.

The Proteases of Plants (V).

BY

S. H. VINES, F.R.S.

Sherardian Professor of Botany in the University of Oxford.

EXPERIMENTS WITH SEEDS (*continued*).

IN a previous paper[1] I gave an account of some experiments on seeds, both germinated and ungerminated, which led to the following conclusions: (1) that the ungerminated seeds contained (*a*) a protease that acted immediately on Witte-peptone, and (*b*) one or more proteases that acted more or less slowly upon the reserve proteids of the seeds; further (2) that the germinated seeds all contained a protease that digested fibrin, and that such a protease was, in certain cases, developed in the substance of the ungerminated seed during the experiment. The difference in point of time between the action on Witte-peptone and that on fibrin, led me to infer that probably the two actions were due to two distinct proteases. The seeds used were those of *Phaseolus multiflorus*, *Phaseolus vulgaris*, *Vicia Faba*, *Pisum sativum*, *Lupinus hirsutus*, and *Zea Mais*. All of them, except those of *Lupinus*, are starchy seeds; in *Lupinus*, which proved to be the most active proteolytically, the non-nitrogenous reserve-material consists mainly of the thickened cell-walls of the cotyledons (hemi-cellulose) and also of some fat in the cells.

Since then I have confined my attention to oily seeds, more especially those of the Hemp (*Cannabis sativa*). I may say at once that I have found oily seeds to be much more proteolytically active than starchy seeds; so much so, in fact, that I have experimented almost exclusively with ungerminated seeds. I should explain that I did not find it necessary to remove the oil from the seeds, as it did not appear to interfere with the progress of the experiments. I may add that various samples of Hemp-seed obtained from different sources were used, with but slightly varying results. The temperature of the incubator was 38° C.

[1] The Proteases of Plants (IV); Ann. Bot., vol. xx, 1906, p. 113.

[Annals of Botany, Vol. XXII. No. LXXXV. January, 1908.]

Ungerminated seed of Cannabis sativa.

EXPERIMENT 1. In this case a mixture of crushed seed with water was used; all subsequent experiments were made with extracts of the seed.

15 grms. of crushed seed were placed in each of two bottles with 100 cc. distilled water; a sample of the liquid gave no tryptophane-reaction: to No. 1 nothing was added; to No. 2, 0·2 grm. of well-washed fibrin. After 20 hours in the incubator No. 1 gave distinct tryptophane-reaction; No. 2 gave a marked reaction, and the fibrin had entirely disappeared: 0·5 grm. fibrin was then added to No. 2. After 48 hours' digestion both gave strong tryptophane-reaction; the 0·5 grm. fibrin added to No. 2 disappeared in 48 hours. Subsequently 0·3 grm. fibrin was added to No. 1 and was digested within 24 hours.

These results prove that autolysis proceeded actively during the experiment, and that the mixture was able to digest fibrin: it may be inferred that the resting seed contains both peptonizing and peptolyzing enzymes. It should be explained that, in such experiments as these, it is difficult to demonstrate the actual formation of peptones, for the liquid gives the biuret-reaction to begin with, owing to the presence of albumoses extracted from the seed.

Some of these seeds were germinated with a view to comparative experiments with the ungerminated seeds and the seedlings; but they were not pursued when the seeds were found to be so active proteolytically. The only observation made was that a watery extract of the seedlings, a week old, gave a strong tryptophane-reaction.

EXPERIMENT 2. 10 grms. of seed were crushed with 100 cc. distilled water, and the same quantity with 100 cc. 2% NaCl-solution: the mixtures were left to filter in the cold all night. The filtered extracts were found to be acid, and to give a precipitate of proteid on boiling, rather more in the NaCl-extract; the NaCl-extract also gave a faint tryptophane-reaction.

About 30 cc. of each extract were put separately into each of two bottles; to one of the H_2O-extract bottles (1) and to one of the NaCl-bottles (3) nothing was added but some HCN (about 0·1%) as an antiseptic; to the other H_2O-bottle (2) and to the other NaCl-bottle (4) 0·2 grm. fibrin was added. After 24 hours' digestion the fibrin in the NaCl-bottle had disappeared; and after 48 hours that in the H_2O-bottle had disappeared. The precipitate on boiling the liquids was observed to diminish gradually. At the close of the experiment (96 hours) the precipitate was very slight in the two H_2O-bottles, rather more in the two NaCl-bottles; the tryptophane-reactions were distinct in all the bottles, except No. 4, in which it was strong.

The diminution of the precipitate on boiling affords evidence of digestive action on the coagulable proteids dissolved in the extract, that is of autolysis. The results confirm those of Experiment 1, and further show that the proteases of the seed can be dissolved out by both water and dilute NaCl-solution, apparently rather more by the latter than by the former.

In order to make sure that the more rapid action on fibrin of the NaCl-extract was not due to saline digestion, a control-experiment was made: 0·2 grm. fibrin was placed in a bottle with 70 cc. of 2% NaCl-solution; after remaining in the incubator for six weeks the fibrin was found to be quite unaltered.

EXPERIMENT 3. The question as to the greater solubility of the fibrin-digesting protease in NaCl-solution was further investigated by the method of preparing a relatively stronger H_2O-extract.

10 grms. of seed were extracted with 100 cc. 2% NaCl-solution, and 20 grms. with 100 cc. distilled water: 50 cc. of each extract were put into separate bottles with 0·2 grm. fibrin and a little HCN. After 24 hours the fibrin in the NaCl-bottle was broken up, that in the H_2O-bottle being unaltered; after 48 hours the fibrin had nearly disappeared in the former, and was breaking up in the latter; after 72 hours there was still some left in the former, and but little in the latter; after 96 hours the fibrin had completely disappeared in both. The tryptophane-reaction was strong in both, but distinctly stronger in the H_2O-bottle; neither liquid gave any appreciable precipitate on boiling. The interesting observation was made that the H_2O-liquid gave only a trace of biuret-reaction, whereas that of the NaCl-liquid was as strong as at the beginning of the experiment. Subsequent special experiments on autolysis confirmed this result (see Experiment 5, p. 107).

It appears from this experiment that the fibrin-digesting activity of the NaCl-extract was about the same as that of the H_2O-extract of twice the strength; the digestive action of the former, though more rapid at first, was eventually overtaken by that of the latter, so that the complete disappearance of the fibrin occurred at about the same time in both. It may be concluded that a 2% solution of NaCl dissolves about twice as much of the peptonizing protease from the seed as does an equal proportion of distilled water. But the possibility still remains that the greater digestive activity of the NaCl-extract may be due, at least in part, to the direct action of the salt upon the process of digestion. The following experiment was made for the purpose of determining this point:—

EXPERIMENT 4. 20 grms. of crushed seed were extracted with 125 cc. H_2O, and 10 grms. with half the quantity of 2% NaCl-solution.

Three bottles, each containing 40 cc., were prepared as follows:—No. 1, H_2O-extract; No. 2, H_2O-extract to which was added 0·8 grm. NaCl (= 2%); No. 3, 2% NaCl-extract: to each bottle were added 0·2 grm. fibrin and a few drops of HCN.

After 24 hours' digestion in the incubator the fibrin in No. 1 was apparently unaltered; in No. 2 it was breaking up; in No. 3 it had nearly all disappeared. 24 hours later the fibrin was breaking up in No. 1; it was about half gone in No. 2; and had entirely disappeared in No. 3. 24 hours later it was reduced in No. 1, and had nearly disappeared in No. 2. 48 hours later the fibrin had not quite gone in No. 1, but had entirely disappeared in No. 2. Hence the fibrin was digested in No. 3

within 48 hours; in No. 2 within 120 hours; in No. 1 digestion was not complete in 120 hours.

It is clear that the presence of the added NaCl in No. 2 promoted digestive activity, the salt apparently playing the part of an 'activator'. But since digestion was still more rapid in the NaCl-extract, it may be concluded that a considerable proportion of the greater activity of NaCl-extracts of seeds, as compared with H_2O-extracts, is to be attributed to the presence of a larger proportion of fibrin-digesting protease in the former than in the latter, enough to justify the inference that the fibrin-digesting protease is more soluble in NaCl-solutions than in distilled water.

The four experiments described suffice to prove that the ungerminated seed of the Hemp contains proteases which are capable (1) of digesting fibrin, and (2) of digesting the reserve-proteids of the seed (autolysis), as shown by the diminution of the precipitate on boiling, and by the development of the tryptophane-reaction. As might be expected, it was found in many experiments that Witte-peptone, when added to the liquids, was readily peptolyzed, as indicated by a more or less strong tryptophane-reaction. It should be mentioned that the extracts sometimes gave a tryptophane-reaction to begin with; a 10% H_2O-extract gave at most a faint reaction, whereas the reaction of 20% H_2O-extract or of a 10% NaCl-extract was distinct.

I did not devote much attention to the investigation of the effect of increasing the acid reaction by the addition of acid, or of diminishing the acidity, or of an alkaline reaction, by adding alkali, a matter that I have laid stress upon in previous papers. One such experiment is, perhaps, worth recording.

Experiment 5. 50 grms. of crushed seed were extracted with 350 cc. distilled water: about 250 cc. of filtrate were obtained; this liquid gave a dense precipitate on boiling, faint tryptophane-reaction, and distinct biuret-reaction. Six bottles, each containing 40 cc. were prepared as follows:—No. 1, extract alone; No. 2, added Na_2CO_3 to 1·25%; No. 3, added HCl to 0·05%; Nos. 4, 5, and 6 were exactly as Nos. 1, 2, and 3, except that 0·2 grm. of fibrin was added to each; HCN was added to all to 0·1%; Nos. 2 and 5 were distinctly alkaline. After 24 hours' digestion, the tryptophane-reactions were, Nos. 1 and 4, marked; Nos. 2 and 5, faint; Nos. 3 and 6, strong; the fibrin in No. 4 was visibly attacked, in No 5 it was swollen and gelatinous, in No. 6 it was quite broken up.

After 48 hours' digestion, the fibrin had almost disappeared in No. 6, and was broken up in Nos. 4 and 5. The reactions of Nos. 1, 2, and 3 was as follows:—

	Tryptophane.	*Boiling.*	*Biuret.*
No. 1,	marked	slight ppt.	scarcely perceptible
„ 2,	very faint	scarcely any	distinct
„ 3,	marked	„	none.

After 72 hours the fibrin had almost entirely disappeared in Nos. 4, 5, 6; the reactions were:—

	Tryptophane.	*Boiling.*	*Biuret.*
No. 1,	strong	very slight ppt.	none
„ 2,	very faint	no ppt.	distinct
„ 3,	strong	turbidity	none
„ 4 (fibrin),	strong	slight ppt.	fair
„ 5 „	very faint	no „	distinct
„ 6 „	strong	no „	fair.

From this it appears that fibrin-digestion was not materially interfered with by the addition of either acid or alkali. The effect on autolysis could not be gauged by the presence or absence of a precipitate on boiling, inasmuch as the native proteids were converted into acid-albumin or alkali-albumin; but it is clear that peptolysis was prevented by alkalinity, since the liquids containing Na_2CO_3 gave but faint tryptophane-reaction, whilst retaining the biuret-reaction which disappeared in the two other liquids (Nos. 1 and 3). Thus the results, so far as they go, are differential as between peptonization and peptolysis in the presence of alkali.

This experiment, however, is not only of interest in this respect, but also in that it confirms the previous observation (Experiment 3, p. 105), that the biuret-reaction disappears during the autolysis of H_2O-extracts, and persists in NaCl-extracts; this result was further established by other experiments on autolysis, of which the following are examples:—

Experiment 6. 20 grms. crushed seed were extracted with 200 cc. 2% NaCl-solution; and 20 grms. with 100 cc. H_2O: 50 cc. of each filtered extract were placed separately in two bottles, to which some HCN was added: both extracts were acid, the H_2O-extract being darker in colour and less clear than the NaCl-extract; their reactions were:—

	Boiling.	*Tryptophane.*	*Biuret.*
NaCl-ext.	dense ppt.	faint	good
H_2O „	less „	distinct	„

After 48 hours' digestion the reactions were—

NaCl-ext.	slight ppt.	distinct	good
H_2O „	rather more ppt.	marked	none

The experiment extended over six days; at the close the reactions were—

NaCl-ext.	very little ppt.	marked	good
H_2O „	slight „	„	none.

In another experiment, Witte-peptone was added to the extracts with the object of ascertaining whether the H_2O-extract was capable of peptolyzing proteids in addition to those formed in autolysis.

Experiment 7. 20 grms. of crushed seed were extracted with 200 cc. H_2O, and 20 grms. with 200 cc. 2% NaCl; the filtered extracts both gave a considerable precipitate

on boiling, distinct tryptophane-reaction, and good biuret-reaction. About 50 cc. of the H_2O-extract were put into each of two bottles, to one of which 0·1 grm. of Witte-peptone was added, and two similar bottles of NaCl-extract were prepared; both the bottles to which Witte-peptone had been added gave strong biuret-reaction; some HCN was added to all the bottles. After 48 hours' digestion the reactions were :—

	Tryptophane.	*Boiling.*	*Biuret.*
H_2O-ext. without W.-peptone	marked	no ppt.	indistinct
NaCl „ „ „	less marked	slight ppt.	good
H_2O „ with „	strong	no „	distinct
NaCl „ „ „	„	slight „	good.
At close of experiment 4 days later—			
H_2O-ext. without W.-peptone	marked	no „	none
NaCl „ „ „	„	„ „	good
H_2O „ with „	strong	„ „	faint
NaCl „ „ „	„	„ „	good.

The marked reduction in the intensity of the biuret-reaction in the bottle to which Witte-peptone had been added proves that the H_2O-extract was able to peptolyze more proteid than it originally contained. Without this experimental addition of proteid the results of autolysis might have been attributed to the larger amount of proteid in solution in the NaCl-extracts as compared with the H_2O-extracts; but it is now made clear that the difference in the biuret-reactions of the two extracts is due to the difference in the relative amount or activity of the peptolyzing enzyme, and not in the relative amounts of proteid that they contain. Whilst NaCl-extracts, on the one hand, digest fibrin more actively on account of the larger amount of the protease that they contain, H_2O-extracts, on the other, are more active peptolytically. Consequently these results bear upon the question that I have raised in several papers, the question as to the number and nature of the proteases occurring in plants: to this I return in the latter portion of this paper.

Experiments with other oily seeds.

I have devoted so much time to the investigation of the Hemp that it has not been possible to make more than a few cursory observations on other oily seeds, those of the Mustard (*Sinapis alba*), the Hazel (*Corylus avellana*), the Castor-Oil plant (*Ricinus communis*), and the Flax (*Linum usitatissimum*).

Sinapis alba. Experiments on autolysis made with aqueous mixtures or extracts (10%) of ungerminated seeds gave negative results, nor did they digest fibrin; but a stronger extract, whether aqueous or of NaCl 2% solution, proved active.

Experiment 1. 15 grms. ground seed were extracted with 100 cc. H_2O, and the same weight with 100 cc. 2% NaCl-solution; the filtrates gave no tryptophane-

reaction; bottles, of 30 cc. each, were prepared as follows: No. 1, H_2O-extract alone; No. 2, 30 cc. H_2O-extract with 0·3 grm. Witte-peptone and 0·2 grm. fibrin; No. 3, NaCl-extract alone; No. 4, NaCl-extract with 0·3 grm. Witte-peptone and 0·2 grm. fibrin: some HCN was added to each bottle. After 24 hours' digestion the results were:—

	Tryptophane-reaction.	*Fibrin.*
No. 1,	faint	
„ 2,	distinct	attacked
„ 3,	„	
„ 4,	marked	gone.

0·3 grm. fibrin was put into No. 4: after 24 more hours' digestion—

No. 1,	marked	
„ 2,	strong	nearly gone
„ 3,	marked	
„ 4,	strong	gone.

These results afford evidence of autolysis, of fibrin-digestion, and of the peptolysis of Witte-peptone; fibrin-digestion was, as usual, more actively carried on by the NaCl-extract.

I found that seeds which had been kept moist for about two days at room-temperature gave more active extracts; and that an extract of seedlings three days old digested fibrin rapidly.

Corylus avellana. 20 grms. of ground kernels were put into each of two bottles with 80 cc. distilled water, and to one bottle 0·2 grm. fibrin was added. The fibrin gradually diminished, and at the end of 72 hours there was scarcely any left; both bottles then gave marked tryptophane-reaction.

Ricinus communis. 10 grms. ungerminated seed (without testa) were extracted with 100 cc. H_2O, and a similar weight with 100 cc. 2% NaCl-solution: both filtered extracts gave a precipitate on boiling; about 80 cc. of each were respectively put into bottles with 0·2 grm. fibrin and some HCN. After 72 hours' digestion the fibrin had disappeared in the NaCl-bottle, that in the H_2O-bottle was unaltered; the contents of the former gave distinct tryptophane-reaction, those of the latter none; the contents of both still gave a precipitate on boiling. Four days later, after a week's digestion, the fibrin in the H_2O-bottle was breaking up, and the liquid in the bottle gave a distinct tryptophane-reaction; the tryptophane-reaction of the contents of the NaCl-bottle was now marked; the H_2O-extract gave only turbidity on boiling, but the NaCl-extract still gave a precipitate.

I found that both H_2O and NaCl-extracts of germinated seeds (10 days) digested fibrin within a week, the NaCl-extract the more rapidly.

Linum usitatissimum. 20 grms. ground ungerminated seed were extracted with 200 cc. distilled water, and a similar weight with the same quantity of 2% NaCl-solution. 40 cc. of the filtered H_2O-extract were put into each of two bottles, to one of which 0·2 grm. of fibrin was added, and similarly with the NaCl-extract: HCN to 0·1% was added to each bottle; the extracts gave a trace of tryptophane-reaction

After 48 hours' digestion the fibrin in the NaCl-bottle had disappeared, and that in the H_2O-bottle was attacked; the contents of the NaCl-bottle gave a faint, those of the H_2O-bottle a distinct, tryptophane-reaction. Three days later the fibrin in the H_2O-bottle had disappeared. At the close of the experiment, after 10 days' digestion, the tryptophane-reactions were:—

H_2O-extract	with fibrin	strong
„	without fibrin	marked
NaCl	with fibrin	„
„	without fibrin	„

These experiments, incomplete as they are, show that all the oily seeds investigated either contained to begin with, or developed during the experiment, proteases that effected both peptonization and peptolysis, and proved themselves to be more proteolytically active than starchy seeds; but they were all much less active than Hemp-seed. There is, however, the serious difficulty in comparing the seeds in this way, that I had no means of knowing how old they were: accurate comparative results can only be expected when the seeds compared are known to be of the same harvest. It is well known that old seed does not germinate so well as new, and probably it is not so active proteolytically; in fact the capacity for germination may depend to some extent upon the presence of active proteases.

The Separation of the Proteases.

In the series of papers on the proteases of plants that I have published in previous volumes of this periodical (1903 to 1906), I have shown that a peptolyzing protease, an ereptase, is generally present, perhaps universally, in the tissues of plants. In some cases—for instance, ordinary foliage-leaves—it appeared that this was the only protease present, since the extract did not digest fibrin. In many cases, however, the extracts not only peptolyzed Witte-peptone, as indicated by the development of the tryptophane-reaction, but digested fibrin as well. The question remained—what is the nature of this fibrin-digesting protease? is it a tryptase or a peptase? In my last paper (April, 1906) I pointed out that though my experiments up to that time did not afford evidence to prove that there is no such thing as 'vegetable trypsin', yet they sufficed to prove that 'vegetable trypsin' is a mixture of proteases, and that ereptase is one of the constituents; and I expressed the opinion that it seemed more probable that the fibrin-digesting constituent was a peptase rather than a tryptase.

In the course of this year I have endeavoured to contribute something to the definite settlement of this vexed question. My idea was to obtain extracts which should contain only either the ereptase or the fibrin-digesting protease. It is, of course, quite easy to obtain an extract containing only ereptase; almost any leaf, bulb, or seed, extracted quickly with a relatively

large quantity of water, will yield an active solution. It is the fibrin-digesting extract that offers difficulties.

Whilst experimenting with Hemp-seed, it struck me that this would be suitable material for the attempt to isolate the proteases. It is unnecessary to give all the tentative efforts that were made. It will suffice to say that I was guided by the fact with which I had long been familiar, and which is especially brought out by the experiments described in the first part of the present paper, that the fibrin-digesting protease is more soluble in NaCl-solutions than in water. Moreover, I was aware that NaCl-solutions also extract a great deal of proteid from seeds; it therefore seemed to be probable that the precipitation of the proteid in such an extract would carry down with it the fibrin-digesting protease. This probability I have succeeded in realizing. I found it to be necessary to use a strong (10%) NaCl-solution, and to obtain a strong extract holding much proteid in solution. On acidifying such an extract with the least possible quantity of acetic acid, a dense precipitate of proteid was formed. Filtering this off, the acid filtrate was found to peptolyze actively, and to have no action on fibrin; the fibrin-digesting protease evidently remained in the precipitate on the filter. Washing the precipitate with 10% NaCl-solution, slightly acidulated with acetic acid, the washings were found at first to act upon Witte-peptone, but this action gradually diminished and eventually ceased. A portion of the washed precipitate was then extracted with distilled water and filtered; the somewhat opalescent filtrate was found to digest fibrin actively, and not to act upon Witte-peptone, as indicated by the absence of the tryptophane-reaction. Further details are given in the following account of typical experiments:—

50 grms. crushed Hemp-seed were extracted with 250 cc. 10% NaCl-solution, and left to filter all night at a low temperature. It may be remarked that, as filtration is slow, it is necessary that the temperature should be as low as possible during the process. The filtrate was a rather viscid liquid, giving dense precipitate on boiling, also a strong tryptophane-reaction, and digesting fibrin actively. To this filtrate acetic acid was now added to 0·2%, and a dense precipitate was produced; the liquid was then put to filter in the cold.

The *acid filtrate* gave turbidity on boiling, and marked tryptophane-reaction: its digestive properties were tested as follows:—40 cc. were placed in each of three bottles: to No. 1, no proteid was added; to No. 2, 0·1 grm. fibrin; to No. 3, 0·2 grm. Witte-peptone. After 24 hours' digestion in the incubator the tryptophane-reactions were:—No. 1, marked; No. 2, marked; No. 3, strong; the fibrin in No. 2 was unaltered. The experiment with No. 2 was continued for three days longer, at the end of which time the fibrin still remained unaltered. Thus the acid filtrate digested Witte-peptone but not fibrin.

The *precipitate produced by acetic acid* was washed on the filter with 100 cc. 10% NaCl-solution containing 0·2% acetic acid, and the digestive activity of the

filtered washings was tested: 40 cc. of the liquid, which gave no tryptophane-reaction, were put into a bottle with 0·2 grm. of Witte-peptone; after 24 hours' digestion the liquid gave distinct tryptophane-reaction. Consequently the precipitate on the filter was again washed with 100 cc. 10% NaCl-solution containing 0·2% acetic acid, and the washings were tested: 40 cc. were put into a bottle with 0·2 grm. Witte-peptone; no tryptophane-reaction was observable after 24 hours' or after 48 hours' digestion; it was therefore concluded that all the ereptase had been washed out of the precipitate.

It now remained to ascertain if the precipitate contained a fibrin-digesting protease. 2 grms. of the precipitate (which was kept in the cold all the time) were treated with about 70 cc. distilled water, and the mixture put to filter. Filtration soon became very slow; the filtrate was turbid, and seemed to continue precipitating; 40 cc. were put into a bottle with 0·5 grm. fibrin, and 30 cc. into another bottle with a solution of 0·2 grm. Witte-peptone in 10 cc. distilled water that had been boiled and filtered; after 24 hours' digestion the fibrin was seen to be much broken up, and in 72 hours it had entirely disappeared: at the end of this time the liquid to which Witte-peptone had been added gave no trace of tryptophane-reaction, nor did the other.

This last experiment was repeated with a more dilute solution: 1 grm. of the precipitate was treated with 50 cc. distilled water; 20 cc. of the filtrate were put into each of two bottles, to one of which 20 cc. distilled water and 0·5 grm. of fibrin were added, to the other 20 cc. of a boiled and filtered solution of 0·2 grm. of Witte-peptone; after 48 hours' digestion the fibrin was disintegrated, though it had not altogether disappeared, in the one bottle, and the contents of the other gave no tryptophane-reaction. Thus a very small quantity of the washed acetic acid precipitate sufficed to give a solution that readily digested fibrin.

In another experiment with the precipitate, 2 grms. were extracted with 80 cc. distilled water, and filtered through muslin instead of through filter-paper. The filtered liquid gave no precipitate on boiling, and no biuret-reaction: 35 cc. of the solution were put into each of two bottles, after boiling in one case; to each 0·2 grm. fibrin was added. After 24 hours in the incubator the fibrin had nearly disappeared in the unboiled liquid, which now gave a strong biuret-reaction; the fibrin in the boiled liquid was unaltered, and the liquid gave no biuret-reaction: 0·4 grm. of fibrin was now added to the unboiled liquid; in 24 hours the fibrin was quite broken up, and neither liquid gave tryptophane-reaction, nor did the boiled liquid give a biuret-reaction.

To return to the *acid filtrate*: I endeavoured to obtain from this a precipitate which, on being dissolved, would give a solution that would act on Witte-peptone. About 130 cc. of it were poured into twice the volume of strong alcohol; a precipitate was formed, and the liquid was filtered. 1 grm. of the precipitate was treated with 50 cc. distilled water and filtered; the filtrate gave no tryptophane-reaction: 20 cc. of it were put into each of two bottles, and to one of them 0·1 grm. Witte-peptone was added. After 24 hours' digestion the contents of the latter gave a faint tryptophane-reaction, which had become quite distinct after 48 hours; the contents of the other bottle gave no reaction.

In all these experiments HCN, 0·1%, was the antiseptic.

The results of these experiments can be very briefly summarized. I have succeeded in isolating from a vegetable tissue, I believe for the first time, a protease that is essentially peptic in its properties, digesting fibrin to albumose or peptone, but not acting on albumose or peptone whether produced by its own digestion of fibrin or added as Witte-peptone. The facts justify the conclusion that the Hemp-seed contains two proteases, the one a peptase, the other an ereptase. What now remains to be done is to apply this method of investigation, modified according to circumstances, to other cases, and so to arrive at a general conclusion as to the nature of 'vegetable trypsin'. With this work I am now occupied.

NOTES.

REDISCOVERY OF STATICE ARBOREA AND DISCOVERY OF A NEW, ALLIED SPECIES.—Since the publication of my articles on the Statices of the *Nobiles*-section in these Annals (vol. xx, nos. lxxviii and lxxix), one species, *Statice arborea*, of that section which had been all but lost, has been rediscovered, thanks to the persevering efforts of Dr. G. V. Perez, of Orotava.

1. Of *Statice arborea* I said, l. c., p. 212, that it 'inhabited at some time within the last 110 years two very small areas on the north coast of Teneriffe, one at the Burgado Cove, the other at Daute, whilst a third equally small area on El Freyle harbours still a much stunted form of it.' There can be no doubt that it has actually disappeared from the Burgado Cove, and it is the Daute locality where *Statice arborea* has been rediscovered. It was found here by Broussonet early in the last century, and as the species was described by Willdenow from his material, Daute is the *locus classicus* of the species. In August of last year specimens, collected by a shepherd on some precipitous rocks just below the hamlet of the 'Tanque Bajo,' not far from Daute, and known as 'Gateadero,' were brought to Dr. Perez, who kindly communicated them to Kew. This year he received further material from the same locality. This time it did not come direct from there, but was taken from a plant which five years ago had been transferred from 'Gateadero' to the garden of a peasant, Domingo Reyes, at Genovás, not far from Icod. The preservation of the species in that locality is no doubt due to its inaccessibility, even for goats. The specimens had, in fact, to be hauled up by ropes to which hooks were attached. Dr. Perez observes that the specimens which he first received resemble much the form *fruticans* from El Freyle, but for the leaves which were 2–3 times larger, whilst that from the cottage garden at Genovás represents the typical arborescent form, and is 'at present (June, 1907) 1·57 m. high and 3 m. in circumference.'

2. Last August Dr. Perez received plants of a *Statice* from Masca, on the west coast of Teneriffe, which he had not seen before, and which he suggested might be *Statice Preauxii* (see ANNALS OF BOTANY, vol. xx, p. 308). He very generously sent them to Kew. From a photograph, which Professor Baccarini Pasquale, of Florence, made for me of the original of *Statice Preauxii* in Webb's herbarium, and from his notes, it is perfectly clear that the *Statice* from Masca resembles very much *Statice Preauxii* in general appearance, but also that they are specifically distinct, and the former is therefore a new species. It is a very remarkable addition to the small *Nobiles*-group and apparently as restricted in its distribution as any of them, having so far been found only on a single high rock, known as 'Tabucha,' about three miles from the sea, facing west to south-west, and not far from Masca, which is south of Punta Feno. I propose to call it *Statice Perezii*, after Dr. G. V. Perez, and give below

a short diagnosis. I would only mention in addition that the affinities of *Statice Perezii* and *Statice Preauxii* lie evidently with *Statice arborea*, with which the former at least shares the beautiful blue of its large and dense panicles. They can, however, be easily distinguished by the peculiar shape of their leaves. This is already seen in the remarkably glaucous seedling plants which were raised at Kew from seed communicated by Dr. Perez.

Statice Perezii (sp. nov.), habitu *Staticae Preauxii* simillima, praesertim ob folia longe petiolata late triangulari-vel rhomboideo-ovata basi saepe truncata, sed petiolis basi utrinque in auriculum brevem triangularem productis, inflorescentiae ramulis pubescentibus, bracteis ad ramulorum bases sitis subulato-caudatis ciliatis et calyce pubescente distincta.

OTTO STAPF.

A PRELIMINARY NOTE ON SCLEROCYSTIS COREMIOIDES, B. re Br.—*Sclerocystis* is one of the new genera instituted by Berkeley and Broome from Ceylon specimens. The characters of the genus are 'Capitulum globosum, tomentosum; stipes cylindricus: flocci compositi: cysti elliptici;' and the species 'looks at first like a *Coremium*; head globose, hard, and compact; flocci rigid, compound; cysts elliptic, slightly rugose, sometimes giving out in every direction soft hairs.' The authors add, 'a very singular plant, of which unfortunately the real nature of the fruit is not apparent.' The genus does not seem to have been rediscovered: indeed, it is scarcely probable that anything concerning the real nature of the fungus would ever be deduced from the description. Saccardo follows Berkeley and Broome's arrangement and includes it among the Mucorineae: Schröter (Engler-Prantl, Pflanzenfamilien) does not mention it.

Fortunately there is a specimen in the Peradeniya herbarium, and it is fairly abundant in wet weather at Peradeniya. From these it can be deduced that Berkeley's unnamed figures (Jour. Linn. Soc. Bot. 14 (1875), tab. 10, f. 56) are intended to represent these species, and are not a continuation of the so-called *Eurotium diplocystis* (fig. 55): *a* shows how Berkeley thought the 'cyst' grew at the top of a stalk, *c* shows the 'cyst' covered with radiating hairs, and *b* shows the 'cyst' in a far more natural position, resting on strands of mycelium.

These cysts are small sclerotia, about a millimetre in diameter. They are produced on a white mycelium which spreads more or less in coarse strands over decaying leaves, &c. They are at first white, and then brown, looking when massed together exactly like a sessile *Chondrioderma*. The sclerotia are produced anywhere along the course of a strand, and are at first widely scattered, but ultimately by the copious growth of others they are densely crowded. The mycelium then disappears, leaving the sclerotia free. This species is parasitic, and kills out *Caladium*, *Colocasia*, and artichoke.

Berkeley and Broome's specimen is immature: the developing sclerotia are white, and there is some mycelium present. Evidently when they saw a sclerotium connected with its mycelial strand, they imagined that the latter was a stalk which had been pressed flat in drying; the soft hairs radiating from a 'cyst' are the broken ends of the hyphae which have gone to form the sclerotium. Thwaites 1014, which

they did not venture to name, consists of ripe sclerotia of the same species, and Thwaites 1013, *Tuber zeylanicum*, is another sclerotium. The latter is much larger, about one centimetre in diameter; it is quite common on rubbish heaps, and when planted in damp sand, it produces a white mycelium which runs along the top of the sand and forms sclerotia of the same size as those of *Sclerocystis coremioides*. It may, however, be a different species.

Up to the present it has not been possible to develop a fructification from these sclerotia: in pure sand, the larger develop a mycelium which produces the sclerotium stage again, while the smaller have not yet produced anything. It seems, however, worthy of record that the description of *Sclerocystis coremioides* was based on a complete misapprehension of the nature of the specimen sent from Ceylon by Thwaites, and that the genus *Sclerocystis*, as described, has no real existence.

Cesati established a new genus *Xenomyces*, on a similar production collected by Beccari in Sarawak. He states that it is 'Genus affine *Sclerocystidi* B. & Br., nec minus enigmaticum.' The description suggests that it is another sclerotium, if not the same species as Berkeley and Broome's.

PERADENIYA. T. PETCH.

THE CYTOLOGY OF RHOEO DISCOLOR.—At the suggestion of my teacher, Prof. Marcus Hartog, I made a preliminary study of the cytology of *Rhoeo discolor*, Hance (*Tradescantia discolor*, Hortt.), in 1905. Its inflorescence is a compact double scorpioid cyme, which facilitates the assemblage of sets of consecutive stages. Many fixatives were tried; but Tellyesniczky's mixture (potassium bichromate, 3 grms.; glacial acetic acid, 5 cc.; water to 100 cc.) proved most satisfactory.

Much preliminary work was done; but other duties make it probable that my complete results may long await publication. The two essential points of interest are (*a*) the small number of chromosomes (4–8); (*b*) the small size of the cells, which enables a considerable number in various stages to be seen in a single field under a magnification of 500 diameters. This may make the plant a very useful object to the cytologist—all the more as it is usually in flower all the year round, in hothouses.

W. J. GALLAGHER

(From the Biological Institute of Queen's College, Cork).

KUÁLA LUMPÚR, Federated Malay States,
May, 1907.

A PHOTOELECTRIC THEORY OF PHOTOSYNTHESIS.—The following is a preliminary abstract of an investigation into the nature and mode of formation of the primary products in photosynthesis which has been carried out in my laboratory at intervals during the past three years. Although the general hypothesis underlying this research was formulated some twelve years ago, various circumstances interfered with all but preliminary experiments until 1905, when the investigation was resumed.

The hypothesis I originally formulated to myself (and communicated verbally to my friend, Dr. J. Reynolds Green, F.R.S.) was, briefly, that the light rays absorbed by chlorophyll are transformed by it into electric energy, and that this transformed energy effects the decomposition of carbonic acid (H_2CO_3) in the cell, with the concomitant formation of an aldehyde and the evolution of oxygen.

To prove the validity of this hypothesis it would appear necessary to establish experimentally the truth of the following propositions:—

1. That an aldehyde is present, even though in very small quantity, in all actively photosynthetic tissues, and that the aldehyde is probably formaldehyde.

2. That the amount of formaldehyde present bears a definite relation to the intensity of illumination.

3. That formaldehyde may be synthetically produced from carbon dioxide in presence of water, with evolution of oxygen, by a feeble electric discharge.

4. That differences in electric potential of sufficient intensity occur in all photosynthetic tissues when adequately illuminated.

5. That the light rays absorbed by chlorophyll are those chiefly concerned in the generation of such electric currents.

Reserving complete details for a subsequent publication, in which I shall have the co-operation of my colleagues, Dr. A. W. Titherley, Lecturer on Organic Chemistry, and Dr. F. J. Brislee, Assistant Lecturer in Physical Chemistry, I may at present briefly indicate the results obtained as evidence in support of these five theses.

1. *Formaldehyde is present, though in very small quantity, in all actively photosynthetic tissues*:—

That formaldehyde is a primary product in photosynthesis was first suggested by Baeyer in 1870, and its presence in green tissues has been affirmed and denied by many investigators since his time. Curtius and Reinke (1897) asserted that an aldehyde appeared and disappeared according as the leaf was illuminated or not. In 1902 Pollacci affirmed the presence of formaldehyde in the leaf and reiterated this statement in 1904, but his latest results have been sceptically received.

The experimental proof of any theory that postulates formaldehyde as a primary product in photosynthesis must obviously depend on the existence of a delicate and reliable test for it. A test of this character has recently been published by Mulliken, Brown and French (1904) for a reference to which I am indebted to Dr. A. W. Titherley, who has latterly been associated with me in the chemistry of this problem. A knowledge of this test enabled me to proceed with my work in 1905. If a quite fresh and insolated leaf (e. g. *Tropaeolum*) be cut into small pieces and shaken up with water, the water extract, after filtration, may be shown to contain formaldehyde in the following way. To about 1 cc. of a 5 per cent. solution of gallic acid in absolute alcohol add about 3 cc. of pure concentrated sulphuric acid so that the two layers do not mix, and afterwards allow a small quantity of the filtrate to stream down the side of the test tube. If the proper precautions be taken, the presence of formaldehyde will be indicated by the appearance of a blue-green ring at the zone of contact of the upper and lower liquids. This test has been carefully investigated by Dr. Titherley, and we are quite convinced both of its delicacy and of its reliability.

2. *The amount of formaldehyde present in the leaf bears a definite relation to the intensity of illumination.*

If water extracts be made of leaves (in the way above described) at different hours of the day or after exposure to different intensities of sunlight, other conditions remaining constant, the depth of the colour zone in the above test varies, being most intense when the leaves have been exposed to diffuse light, and feeble or absent when the leaves have been placed either in very weak or very intense light. These results are quite in accord with those obtained by previous investigators and especially by Pantanelli (1904), who found that the optimum decomposition of carbon dioxide took place when the light intensity amounted to one quarter of that of direct sunlight, but that as the light intensity increased photosynthesis decreased, other conditions remaining constant.

3. *Formaldehyde may be synthesized from carbon dioxide in presence of water by feeble electric discharge.*

In my first experiments I used the simple method of saturating distilled water with carbon dioxide gas and passing a current from a dry cell through the solution, using platinum or copper electrodes. In some cases, I thought I was able to detect minute traces of formaldehyde, but notwithstanding several modifications in my apparatus I failed to convince myself of its constant occurrence. My colleague, Dr. F. J. Brislee, directed my attention to Loeb's paper (Zeit. f. Elektrochemie, 1906), and by employing his apparatus and a silent electric discharge, the presence of formaldehyde could be demonstrated with the gallic-sulphuric test in every case. Loeb, in discussing the bearing of his results on photosynthesis, holds that formaldehyde becomes by polymerization glycolaldehyde, which readily undergoes transformation into a sugar, but holds that the function of the chlorophyll is to remove the oxygen and at the same time to absorb the carbon dioxide, acting physiologically in an analogous but reverse manner to haemoglobin.

4. *Electric discharges of sufficient intensity occur in photosynthetic tissues when adequately illuminated.*

The researches of Kunkel (1882), Haake (1892) and others have demonstrated the existence of electric currents in green plant-organs, and Klein's (1898) investigations have shown that these currents are subject to regular variations according to the degree of illumination to which the parts are subjected. It would seem, however, that Klein considers the electric currents as something apart from the light and merely influenced by it; according to the theory here proposed the electric currents are the expression of the transformation of the rays absorbed by the chlorophyll.

5. *The light rays absorbed by chlorophyll are those specially concerned in the generation of the electric currents demonstrable in photosynthetic tissues.*

Nagamatsz (1886) and, more recently, Griffon (1900) have shown that no photosynthesis takes place in a leaf illuminated by light which has passed through other leaves (indicated in their experiments by absence of starch). I have confirmed this by other methods and found that no formaldehyde can be demonstrated in an extract of a leaf which has been exposed to light which has passed through another, although the upper leaf shows the presence of formaldehyde quite distinctly.

Again, if a leaf be illuminated by light which has passed through another leaf, the current at once ceases, reappears if illuminated by blue-violet light, and becomes nearly as great when illuminated by red light as it is when illuminated by white light.

Meldola in his Presidential Address to the Chemical Society (1906) says:—'From formaldehyde to fructose the laboratory evidence is fairly complete. It remains only to connect formaldehyde with carbonic acid by some photolytic method which may be regarded as above suspicion—and the discovery of such a method may be looked for sooner or later—in order to say that the chain of chemical evidence is quite complete.'

In our forthcoming paper the detailed evidence will be given on which we believe that we are justified in claiming that our work fills the gap indicated by Prof. Meldola in the sentences quoted; the present note is merely an outline of the chain of evidence and does not touch on various collateral problems which have arisen in the course of the investigation.

R. J. HARVEY GIBSON.

UNIVERSITY OF LIVERPOOL.

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The Reconstruction of a Race of Primitive Angiosperms.

BY

ETHEL SARGANT, F.L.S.

With twenty-one Figures in the Text.

THE origin of Angiosperms is perhaps the most important problem which botanical morphology has yet to solve. The advance of knowledge, which has thrown light on so many questions formerly to all appearance insoluble, seemed for a long time rather to obscure than to illuminate this subject. But of late some progress has been made. New facts have been contributed to the scanty evidence hitherto available, and several botanists have attempted to collect and piece together all such evidence, scattered, fragmentary, and insufficient as it is.

On the one hand, our knowledge of the origin and development of the embryo-sac, both in Angiosperms and Gymnosperms, is more extensive and more precise. The structure of Angiospermous seedlings has been examined from a phylogenetic standpoint.[1] Dr. Wieland has described the bisexual strobili of the American Bennettiteae, which offer so many points of resemblance to the flowers of Angiosperms (89).

On the other hand, we have treatises and speculations on the general question. The Morphology of Angiosperms (Coulter and Chamberlain, 19) is written throughout from a phylogenetic standpoint. The vexed question of the comparative antiquity of Monocotyledons and Dicotyledons has been revived on taxonomic (Hallier, 34) as well as on biological and anatomical grounds.[2] The origin of the Angiospermous flower has been discussed.[3] Finally, in a recent paper on the origin of Angiosperms (4), Messrs. Arber and Parkin have sketched out a possible line of descent for Angiosperms from Pteridosperms.

The evidence thus brought together falls naturally under two heads. On one side we have an enormous mass of morphological detail concerning living Angiosperms—at once the largest, the best known, and the most

[1] Sargant (72); Tansley and Thomas (87); Anonymous (2); T. G. Hill (40, 41); A. W. Hill (39).
[2] Henslow (38); Balfour (8); Lyon (57, 58); Sargant (72, 73).
[3] Benson (9); Robertson, C. (68); Oliver, F. W. (62); Scott, D. H. (80).

[Annals of Botany, Vol. XXII. No. LXXXVI. April, 1908.]

highly differentiated group in the vegetable kingdom. Their classification has occupied many generations of botanists, and the result is no doubt in the main a truly natural system ; that is to say, a system based on real affinities. With such wealth of evidence to draw upon, we might expect to work backwards with certainty from the most primitive forms which we know to their common ancestors—a race which of course has been long extinct. But this is so far from being the case that authorities are still hopelessly divided on the interpretation of the facts.

On the other side is all we know about the forms, living or extinct, which may have stood near the main line of Angiospermous descent. The difficulty in this case is want of evidence. The floral structure of Angiosperms by itself isolates them from all other groups, without taking other characters into consideration.

In the most recent attempt to reconstruct the pedigree of Angiosperms (Arber and Parkin, 4, p. 77)[1], the authors start from a Palaeozoic ancestor, which they place among the newly recognized group of Pteridosperms. They trace the development of the flower through a series of hypothetical forms—pro-anthostrobilus (p. 63, Fig. 4) ; eu-anthostrobilus (p. 44, Fig. 1)—up to the more complex form of Ranal flower, *Magnolia* or *Liriodendron*. While the female sporophyll of the pro-anthostrobilus is suggested by that of *Cycas*, the whole scheme of the fructification is intermediate between the Angiospermous flower, on the one hand, and the bisexual strobili described in the American Bennettiteae by Dr. Wieland, on the other. The eu-anthostrobilus makes a much nearer approach in detail to the Ranal type, considered by the authors as the most primitive form of flower now in existence.

It will be seen that even this bare outline of a possible pedigree for Angiosperms depends largely on two recent additions to our knowledge: on the recognition of the Pteridosperms as a group of primitive seed-plants, and on the description of bisexual strobili among the Bennettiteae.

The memoir (4) was not published in full until July, 1907, but it was communicated to the Linnaean Society at the meeting on March 21 of that year, when the authors read an abstract which gave rise to a discussion on the Origin of Angiosperms (3, p. 13).

On the earlier date I was still engaged in preparing a course of lectures on the Ancestry of Angiosperms, which were delivered for the London University in May and June, 1907. The aim of the course was to reconstruct the common stock from which I assumed Monocotyledons and Dicotyledons to be descended. This stock was referred to as the Primitive Angiosperm. The subject proved sufficient to occupy eight lectures without entering at any length into the vexed question of the primitive flower.

[1] The Table of Relationships here quoted is reproduced, by kind permission of the authors, on p. 137 of this memoir.

The present paper is an abstract of those lectures. It may be considered as a complement to that part of Messrs. Arber and Parkin's memoir which deals with the immediate predecessors of our living Angiosperms. The evidence brought together in that memoir relates mainly, though not exclusively, to floral structure. I am in complete agreement with the general conclusions reached by its authors. Accordingly, I have attempted no separate reconstruction of the flower of the Primitive Angiosperms.

The evidence considered in the present paper, then, is in the main derived from the study of living Angiosperms. This evidence will be used to reconstruct a comparatively recent race of plants—the latest ancestors which Monocotyledons and Dicotyledons had in common. In dealing with their floral structure Messrs. Arber and Parkin's results will be accepted as a working hypothesis.

Before discussing the evidence on which we may hope to reconstruct the Primitive Angiosperms, it is essential to inquire whether such a race ever existed. Do all our living Angiosperms spring from a common stock? If not—if they have been derived from different descendants of the original Pteridosperm, at several epochs and through distinct lines of descent—the problem becomes very much more complicated. Indeed, in the present state of our knowledge it could not be profitably attacked.

The argument in favour of the monophyletic origin of Angiosperms may be briefly summarized thus: The characters which Monocotyledons and Dicotyledons have in common are too numerous and too uniform to have been acquired independently in response to similar conditions. They must be derived by inheritance from a common stock—a race of Angiosperms, since it possessed the characters common to both classes within that group.

This argument appears to me conclusive, but, while the probability of such origin is called in question by botanists of authority,[1] the truth of it cannot be assumed without discussion. The present essay is, therefore, divided and subdivided as follows:—

I. Reasons for believing that Angiosperms are monophyletic.

II. Reconstruction of the primitive race of Angiosperms.

1. Floral structure.
2. Stem anatomy.
3. Number of cotyledons.
4. Minor characters considered in connexion with phylogenetic schemes.

Monophyletic Origin of Angiosperms.

The remarkable isolation of Angiosperms in the vegetable kingdom is due of course to the absence of intermediate forms either living or fossil.

[1] Balfour (8); Coulter (18); Coulter and Chamberlain (19), p. 283.

That such forms were once present is inferred from the sudden appearance of certain characters in this group. Angiosperms possess structures which are unique: for instance, the flower, the carpel, the endosperm. These members are found in Monocotyledons and Dicotyledons alike, but are absent from all other groups. The history of their origin has not yet been recovered: it is not even clear what members represent them in the lower plants.

The flower, the carpel, and the endosperm then have been evolved by Angiosperms and their ancestors since the epoch when they were represented by a race of Pteridosperms. The question is, whether these members appeared in one line of descent or several. Monocotyledons and Dicotyledons must have had a common ancestor at some time, but was that ancestor in essentials an Angiosperm? Did it—to take the simplest test—possess a true carpel?

The problem so stated is reduced to its simplest terms. We suppose that Angiosperms were derived from two races only, one of them giving rise to Monocotyledons, the other to Dicotyledons. We agree to consider for the moment one character only among those peculiar to the two classes, neglecting not only the other two unique characters, but also the numerous minor features which they have in common.

If the common ancestor X did not possess true carpels, then carpels must have been evolved independently by Monocotyledons and Dicotyledons. Naked seeds were transmitted by X to its branches Y and Z. In the course of generations the seeds of both races have become enclosed in a carpel. There is no evidence to show how this occurred. The sporophyll might gradually close round one or more ovules, or some sort of cupule (Oliver, 62) might become a carpel. But though the sporophyll or the cupule is inherited from X, each step in its transformation must have been taken independently by Y and Z, in response no doubt to the demands of the environment.

In this way each race might well acquire a carpellary organ, but the chances are certainly much against the evolution of precisely similar members in both. The Angiospermous carpel combines several functions. It protects the ovule, and also collects pollen-grains and directs the course of their tubes. But in other groups these functions are not always performed by the same structure. In the Bennettiteae the ovules are sheltered by peltate scales, which serve as a very efficient protection to them and also to the seeds. The pollen, however, is still collected by the integument of the ovule, and germinates on the micropyle.

If in response to their surroundings both races developed organs which would at once protect the ovule and secure its pollination, we should still expect to find some constant difference in the carpels of the two races, due to their distinct race-history. But no such difference exists. There are indeed single carpels and carpels united into a pistil; superior and inferior

ovaries; stalked stigmas and sessile ones. But all these forms occur indifferently among Monocotyledons and Dicotyledons. The natural inference from the facts is that carpels were derived by inheritance from an ancestral race common to both classes.

The improbability of a multiple origin is of course very greatly increased if Angiosperms are derived from more than two non-Angiospermous races; that is, if we suppose carpels to have been evolved in three or more parallel lines of descent.

A similar argument applies to two other characters, which are most conveniently treated together, the germination of the embryo-sac, and the formation of the endosperm in Angiosperms. The strength of this argument appears very great when it is considered (1) that the first nuclear divisions within the embryo-sac are highly characteristic of Angiosperms. In other words, they differ in a marked way from the first steps in the germination of any other megaspore. (2) That the origin of the endosperm from a fusion of three nuclei is still more characteristic, and that its morphological value is still unknown. (3) That both processes, though complicated and therefore offering many possibilities of variation, are remarkably uniform throughout the group. The very few exceptions recorded belong for the most part to parthenogenetic or apogamous species, or to forms clearly degraded by parasitism or similar causes. Such forms can hardly be regarded as primitive.

These considerations point to a very long period during which the Angiosperms as we know them now were evolved—a period long enough to stamp such well-defined characters on the race, and also to allow of the disappearance of the numerous intermediate forms which must have existed once.

The discovery of the origin of the endosperm from a fusion of three nuclei is quite recent, and as the literature of the subject is scattered we may briefly discuss the strength of the evidence. Up to 1898 the primary endosperm nucleus was believed to arise from the fusion of the two polar nuclei in the embryo-sac (Strasburger, 84). In that year Nawaschin (61) showed that a third nucleus takes part in this fusion. He was working on *Lilium Martagon* and *Fritillaria tenella*, and in these plants he identified the third nucleus as one of the two generative nuclei derived from the pollen-tube. The other, of course, had fertilized the ovum. It was known that two generative cells entered the embryo-sac, but it had been previously believed that when the nucleus of one had fused with the nucleus of the ovum the second generative cell broke down like the synergids.

Nawaschin's discovery was soon confirmed by the publication of Guignard's independent researches (32). Since 1899 a literature has grown up on this subject. The most recent résumés are those of Guérin (30)

and Ernst (24). The section devoted to the subject in Coulter and Chamberlain's Morphology of Angiosperms (19) gives a list of references up to 1903. In this work, however, the authors express themselves with a caution which even in 1903 appeared exaggerated to many morphologists. They remark on the wide distribution of the phenomenon, but add: 'Probably it is not safe to infer the general occurrence of double fertilization, although the observations already include sixteen families, about forty genera, and over sixty species.' Sixty species is, of course, a very small fraction of the whole number of Angiosperms, but when they represent forty genera and sixteen families of very diverse affinities the evidence covers a good deal of ground. The strongest point in the case, however, is the absence of evidence to the contrary. Excepting apogamous species and anomalous cases of that kind, the endosperm has been found to arise from such a fusion as Nawaschin described wherever its origin has been carefully traced. The partial exception of *Peperomia* will be discussed later.

Evidence is still accumulating in the same sense. My list to the end of 1907 only reaches seventy-eight species, representing fifty-two genera and twenty-two families, but it is no doubt very incomplete. Considering the difficulties of observation, even this is a considerable body of evidence to be collected in nine years. It justifies botanists in assuming that the process of double fertilization is universal among Angiosperms.

Before considering the views now held on the homology of the endosperm, it will be convenient to review the facts very briefly.

The female prothallus of Pteridophytes is formed by the germination of the megaspore, and is fully developed at the period when the oösphere is formed within the archegonium.

The female prothallus—the so-called endosperm—of Gymnosperms arises by division of the nucleus within the megaspore or embryo-sac. Nuclear division is followed after some nuclear generations by cell-division. In the majority of cases the embryo-sac is filled with prothallial tissue before archegonia appear, and therefore this tissue does not develop further after fertilization.

The female gametophyte is quite clearly defined in both groups: its development begins with the germination of the megaspore and ends with the act of fertilization. Moreover, gametophytic tissue cannot be mistaken, because the nuclei within it have only half the number of chromosomes characteristic of sporophytic tissue.

The female gametophyte of Angiosperms undoubtedly begins with the division of the embryo-sac nucleus, for the embryo-sac is acknowledged to represent the megaspore. Three successive mitoses give rise to eight nuclei within the embryo-sac—one of them the nucleus of the ovum. So far we are no doubt dealing with the gametophyte, yet already—in certain genera at any rate—Overton's test fails us. In these genera the double or

sporophytic number of chromosomes appear in an arbitrary way in the chalazal nucleus of the second generation. But we do not know whether this remarkable anomaly is general or even common.

After fertilization two distinct bodies, the embryo and the endosperm, are formed in the embryo-sac, and they shortly fill it to the exclusion of all other tissue. The embryo is derived from the fertilized ovum, and of course belongs to the succeeding generation—the young sporophyte. The endosperm is derived from the union of three nuclei: two belonging to the female gametophyte of the mother plant, and one to the male gametophyte of the plant whose male element has entered the fertilized ovum. The synergids and antipodals disappear sooner or later: there is then no trace left in the embryo-sac of the female gametophyte as it existed before fertilization, and no tissue whose nuclei show the gametophytic number of chromosomes.

The only possible representative of the female gametophyte is the endosperm, and before 1898 most botanists considered it to be a belated female prothallus. This view, though sometimes attributed to Hofmeister, was in fact first maintained by Strasburger (85, pp. 137–139) in 1879.

Le Monnier (53) published an alternative hypothesis in 1887. He regarded the union of the polar nuclei as a sort of fertilization. The upper polar nucleus is sister to that of the ovum: it may well be considered as a female nucleus. The lower polar nucleus must then represent the male element. The endosperm is developed as a result of their union: it is therefore a short-lived misshapen embryo.

Botanists are still divided between these two camps, though Nawaschin's discovery of 1898 has certainly increased the number of Le Monnier's followers. But it is no part of my plan to discuss their rival claims here. The pregnant fact in dealing with the ancestry of Angiosperms is that the question is still undecided. Either view can be held, and is held, by botanists of undoubted authority. In other words, we have so far no links, living or fossil, which enable us to trace the historical development of the endosperm in Angiosperms with any degree of certainty.[1]

Such evidence may be sought in two directions. We may examine the endosperm formation among aberrant Angiosperms, particularly among such forms as we are disposed on other grounds to consider primitive in structure. Or we may seek for some suggestion of the origin of the endosperm in the comparative structure of the Gymnospermous prothallus.

Knowledge of the minute structure of living plants is as yet so far from complete that we may still hope to find an Angiospermous embryo-sac in which the endosperm shows some decided trace of its origin—whether it prove to be a belated prothallus, a specialized sporophyte, or some other

[1] Miss Berridge's interpretation of certain facts recently described in *Ephedra* may perhaps afford a clue. See p. 133.

member. So far, however, this hope has grown fainter with every advance in knowledge.

Starting from the other side of the gulf, much has been done of late years to investigate the origin and development of the male and female gametophyte among Gymnosperms. The variety of structure in allied genera is astonishing, particularly when contrasted with the uniformity of Angiosperms. But while the identity of the food-tissue in the embryo-sac with the prothallus of Pteridophytes is clear in every form, no comparison with the endosperm of Angiosperms is even suggested by the development of such food-tissue, except perhaps among the Gnetaceae.

Accordingly, I propose to discuss first the anomalous embryo-sacs hitherto described among Angiosperms, and then to refer very shortly to the isolated cases of *Gnetum* and *Ephedra*.

The most complete account of the Angiospermous embryo-sac before fertilization is given in Coulter and Chamberlain's Morphology of Angiosperms (19), published in 1903. Guérin's more critical account (30) of 1904 is substantially the same. Both authorities remark on the extraordinary uniformity of this structure. The number of exceptions which they record is certainly very small compared with the number of species investigated. Even this small number should, I think, be considerably reduced, but on the other hand two species of *Cypripedium* must be added (Pace, 63).

Species belonging to five genera are described as showing considerable deviation from the normal type. They are:—

Peperomia pellucida and *P. hispidula*.
Gunnera—several species, but chiefly *G. Hamiltoni*.
Tulipa sylvestris and *T. Celsiana*.
Juglans nigra and *J. regia*.
Trillium grandiflorum.

The anomalies in *Peperomia pellucida* were first described by Campbell (15) in 1899. A fuller and rather different account was published in the following year by Johnson (47), including observations on allied forms. Johnson has quite recently described a second species of *Peperomia* (49). Its endosperm formation shows anomalies similar to those of *P. pellucida*, but with certain interesting departures from that type.

In *Peperomia*, as in *Lilium* and other genera, the embryo-sac mother-cell becomes the embryo-sac without division. In *P. pellucida* sixteen nuclei are formed within the embryo-sac by repeated division of the original nucleus and its descendants. These nuclei are distributed pretty evenly in the peripheral layer of cytoplasm. One near the micropyle becomes invested with a definite mass of cytoplasm and can afterwards be recognized as the nucleus of the ovum. A neighbouring nucleus, which generally lies immediately under the micropyle, is likewise invested by cytoplasm, and

the naked cell thus formed may represent a synergid. Eight nuclei mass together and after fertilization fuse to form the primary endosperm nucleus. The six nuclei which remain retain their parietal position and are cut off by walls from the cytoplasm of the embryo-sac. In the end they are crushed and absorbed by the developing endosperm.

No male nucleus has been identified in the group which becomes the primary nucleus of the endosperm. But this may be due to the absence of the stages which immediately succeed the act of fertilization in the preparations examined. For in all the cases described the male nucleus was about the same size as the nucleus of the ovum with which it was fusing. This indicates that it had entered the ovum some time. A similar nucleus within a group of eight would, of course, be indistinguishable from them.

The history of *P. hispidula* is similar, but not identical (Johnson, 49). In the eight-nucleate embryo-sac, two nuclei are near the micropyle and six at the chalazal end. The succeeding mitosis forms twelve chalazal and four micropylar nuclei. One of the latter becomes a well-defined ovum: the other a synergid. Two micropylar nuclei travel into the centre of the embryo-sac, where they fuse with the twelve chalazal nuclei. This fusion of fourteen nuclei becomes the primary nucleus of the endosperm.

The allied genera, *Piper*, *Heckeria*, and *Saururus*, were found by Professor Johnson (48) to form perfectly normal embryo-sacs, and he concludes that the peculiarities of *Peperomia* must be considered as derived from the usual type rather than primitive. The facts just described support this conclusion. In primitive species we should expect the endosperm to be better developed than in the normal embryo-sac—nearer, that is, to the original structure, whether gametophyte or sporophyte, from which the tissue familiar to us has been reduced. But in both species of *Peperomia* the endosperm in the mature seed consists of forty or fifty cells only, and is of very small bulk compared to the perisperm, which is the effective food-tissue. Indeed all analogy goes to show that a fusion of many nuclei—eight in *P. pellucida* and fourteen in *P. hispidula*—is far more likely to produce a small mass of short-lived tissue than a vigorous body capable of development into a permanent organ.

Peperomia stands alone as a normal deviation from type. For though Schnegg (79) has found similar features in the embryo-sac of *Gunnera*, he gives very strong reasons for thinking that in the species examined the embryo is formed without fertilization. If so, the example is irrelevant. For when the generative nuclei never enter the embryo-sac the origin of the endosperm must be as irregular as that of the embryo, and its formation is likely to be abnormal in other respects.

Of the three genera which remain in the list of exceptions on p. 128, the only one which need concern us is *Tulipa*. In the two species, *T. sylvestris* and *T. Clusiana*, Guignard (33) found that the embryo-sac before fer-

tilization contains eight nuclei, without any trace of cell-formation and without the usual very characteristic orientation. The two which later act as synergids are sometimes distinguished by their smaller size, and the lower polar nucleus is from the first conspicuously different from the rest. The remaining five are precisely similar to each other: no one of them can be picked out as the future egg-nucleus. *Tulipa Gesneriana*, however, has a perfectly normal embryo-sac.

This fact alone would indicate that the peculiarities of the species described can hardly be a survival of primitive characters. Indeed, the deviation from the usual structure is not very great, and may be more apparent than real. The irregular orientation clearly depends largely on the absence of a central vacuole. It does not follow that no difference exists between the five nuclei, which later behave quite normally as egg-nucleus, upper polar nucleus, and antipodal nuclei, because we cannot perceive such difference. To trace the formation of these nuclei in greater detail than Guignard has done would be interesting. In *Tulipa Gesneriana* Ernst has shown that each nucleus in the chalazal tetrad is built up of more chromosomes than go to each of the micropylar tetrad. If this should prove to be the case in *Tulipa sylvestris* and *T. Clusiana* also, three of the five similar nuclei must be marked out as antipodals from their first formation. The egg-nucleus must be one of the two formed with the reduced number of chromosomes.

A similar but less marked absence of polarity is shown in certain exceptional embryo-sacs of *Trillium grandiflorum* according to Ernst (23), and of *Juglans nigra* according to Karsten (50 *a*). But in the large majority of individuals examined both observers found the embryo-sacs nearly if not quite normal. These genera should certainly not be included in such a list as this.

The species of *Cypripedium* described by Miss Pace (63) are *C. spectabile* and *C. parviflorum*, with corroborative sections from two other species. The embryo-sac mother-cell divides once: the upper segment soon perishes, the lower becomes the embryo-sac. After division of its primary nucleus, both daughter-nuclei divide again: the mature embryo-sac contains four nuclei. At the time of fertilization three nuclei are surrounded by delicate cell-walls, and orientated like an egg-apparatus: the fourth lies near the chalaza (63, Figs. 29, 30).

In entering the embryo-sac the pollen-tube seems to push the nucleus of one synergid-like cell in front of it. This nucleus passes to the chalazal end and fuses with the fourth nucleus and with one of the generative nuclei from the pollen-tube. The other generative nucleus fertilizes the ovum in the usual way.

This is an important variant on the normal embryo-sac structure, but clearly a reduction from it. The exact homologies of the nuclei are obscure. If we consider the embryo-sac of *Cypripedium* to represent the upper half of the *Lilium* sac, its four nuclei are truly homologous with the upper tetrad

in *Lilium*. The only variation in the details of fertilization is that the nucleus of one synergid takes the place of the lower polar nucleus.

This explanation seems likely, for the Orchids have always been considered as highly specialized forms, probably derived from ancestors with flowers of liliaceous symmetry. But if the four-nucleate embryo-sac of *Cypripedium* really represents the eight-nucleate sac of *Lilium*, the exact origin of each nucleus becomes important. Is the chalazal nucleus sister to the ovum nucleus, or to one of those in the synergidal cells?

These are the major irregularities hitherto described in normally fertilized embryo-sacs. The minor irregularities chiefly concern the antipodal cells. One of these variations may be of some phylogenetic importance. The antipodals occasionally multiply—in some cases forming quite a tissue at the base of the embryo-sac—either before or after fertilization. This character is common in certain groups, as the Gramineae and Compositae. It has been recorded lately among the Gentians (Guérin, 29), as well as in several isolated genera (Guérin, 30, pp. 39–41); and the tissue thus formed may possibly represent the primitive prothallus or female gametophyte.

Considering the vast number of embryo-sacs described, these anomalies are exceedingly few. Indeed, if we exclude all apogamous and parthenogenetic forms, we find the formation of the ovum and of the endosperm to be alike in every genus examined, with the single exception of *Peperomia*. This is the more surprising, as forms likely to show primitive features have been picked out for examination, and, again excepting *Peperomia*, they have all proved perfectly normal in essentials. Such are *Piper* and *Heckeria* (Johnson, 48), *Casuarina* (Frye, 26), *Carpinus* (Benson, Sanday, and Berridge, 10), and *Drimys* (Strasburger, 86).

In short, the early history of the embryo-sac is wonderfully uniform throughout the Angiosperms. This cannot be attributed merely to the great reduction of the female gametophyte. For the nuclear divisions within the embryo-sac are extremely characteristic in the final orientation of their products. Nor can the uniformity observed be put down to insufficient evidence, for the early history of the embryo-sac has been followed in a great number of species.

The formation of the endosperm is likewise very uniform throughout the group. In every normal case[1] it is undoubtedly formed after fertilization. Its origin from a nuclear fusion has been observed in numberless instances; indeed there is no normal case to the contrary. The regular occurrence of a triple fusion in which one element is a male nucleus has also been recorded in many cases. The only real exception on record is *Peperomia*, where, as just stated, more than three nuclei take part in the fusion, and the presence of a male nucleus within it has not been verified.

[1] By normal case I mean an embryo-sac in which the ovum is normally fertilized.

Thus the comparison of Angiospermous embryo-sacs with each other has given no clue to their primitive structure. What suggestions are offered by Gymnosperms?

Among Gymnosperms, with the exception of *Gnetum* and perhaps *Welwitschia*, the mature unfertilized embryo-sac contains archegonia embedded in prothallial tissue. Archegonia are never found in Angiosperms, and we have seen that the exact equivalent of the prothallus cannot be determined. The absence of archegonia from the embryo-sac of *Gnetum* naturally suggests an approach to Angiospermous structure.

The three genera included in the Gnetaceae differ widely in the structure of their embryo-sac. In *Ephedra*, undoubtedly the most primitive form, it is completely Gymnospermous; but certain features in its development both before and after fertilization have suggested to Miss Berridge (11) a new interpretation of the Angiospermous embryo-sac.

The facts on which this hypothesis is based have been lately published by the same author in conjunction with Miss Sanday (12). They are, shortly, as follows. The upper part of the mature embryo-sac in *Ephedra distachya* is partly divided from the lower region by a constriction in the wall. The tissue which fills it is on the whole looser in texture, more spongy, than that of the lower cavity. But near the apex of this upper region is a 'compact conical mass of archegonia and jacket-cells' (12, p. 129). The initials of archegonia and jacket-cells are alike: they are found as a pyramidal group of tubular alveoli in the young embryo-sac (12, Fig. 3). Each archegonium initial gives rise to the primary neck-cell and the central cell. Within the central cell a nuclear division, apparently amitotic, separates the nucleus of the ovum from the ventral canal nucleus. A ventral canal cell is not formed.

'At the time when the primary neck-cells are being cut off from the initials, the tubular cells between the latter are undergoing a series of divisions, and forming rows of jacket-cells arranged in a regular manner. The likeness of these to the central cells, except in the matter of size, is apparent in Fig. 4.' . . . 'This tissue keeps pace with the growth of the central cell, first by cell division, and later by individual growth of the cells. They soon become crowded with food material, and the nucleus divides into two by direct division long before this occurs in the central cells' (12, p. 167).

The jacket-cell nuclei are capable in *E. distachya* of giving rise to apogamous proembryos after fertilization has taken place in the archegonium they enclose. Before fertilization, nuclear fusions are common in the jacket-cells. A nucleus from one jacket-cell migrates to another and fuses with a nucleus within it. A nucleus may even migrate from a prothallial cell into an adjacent jacket-cell (11, p. 282). Mitotic figures with twenty-four chromosomes—the sporophytic number—are found within the jacket-cells at this

period. The fact that an empty cell is commonly found in the neighbourhood of that in which such mitosis is taking place confirms the conclusion naturally drawn from the number of chromosomes that the dividing nucleus is a fusion product. Apogamous proembryos are probably formed from the daughter nuclei of such mitoses.

The apogamous proembryos may be formed *in situ*; that is, within the jacket-cells to which their primary nuclei belong. In such cases their development rarely proceeds far. But nuclei from the jacket-cells sometimes penetrate the wall which divides them from the central cell of the archegonium, and there is good reason to think that the proembryos found within it are produced by the activity of such escaped nuclei. Such proembryos often develop suspensors, which may even bear rudimentary embryos at their tips (11, p. 282). Since they are always formed after fertilization, their production may depend on some stimulus transmitted from the pollen-tube. Miss Berridge even thinks it possible that an escaped jacket-nucleus may sometimes fuse with a nucleus from the pollen-tube (11, p. 283).

In *Gnetum* the archegonium has disappeared altogether. Karsten (50) and Lotsy (55) have shown that a number of equivalent nuclei lie near the apex of the embryo-sac. Each of these seems to be a female nucleus, and in *Gn. Gnemon* at any rate each pollen-tube which discharges its contents into the embryo-sac fertilizes two of them (Lotsy (55), p. 96 and Fig. 45). Before fertilization there is no appearance of cell-formation round these nuclei. The prothallus fills the lower part of the embryo-sac. In *Gn. Gnemon* it is practically complete before fertilization (Lotsy); but in the species described by Karsten its development is arrested at an early stage, and is not resumed until fertilization of one or more of the apical nuclei has taken place.

Details of the fertilization of *Welwitschia* are still lacking, but Pearson (64) has described the development of the young embryo-sac very fully, and it is clear that cell-formation is complete in every part of it some time before fertilization. Each archegonium must therefore be represented by one cell at least, not, as in *Gnetum*, by a single nucleus. As in *Gnetum*, the apical end of the embryo-sac is clearly differentiated from the lower part.

Miss Berridge suggests that the endosperm in Angiosperms may be comparable to the apogamous proembryos produced within the archegonium of *Ephedra distachya* by division of nuclei escaped from jacket-cells. Her interpretation of the Angiospermous embryo-sac is best given in her own words:—

'Here therefore we have a process occurring in the embryo-sac of a Gymnospermic genus, which shows a remarkable likeness to the development of the Angiospermic endosperm after triple fusion; that is to say, we have cell-formation resulting from the fusion of nuclei, one of which at least is allied to the egg-nucleus, and taking place under the stimulus due to the entry of the pollen-tube.

'Moreover, if the polar nucleus is regarded as the homologue of the jacket-initial nucleus, it is possible to trace among the eight nuclei of the Angiosperm embryo-sac representatives of the four classes of cells which we find in the sac of *Ephedra* and other Gymnosperms.

'1. The egg-nucleus in Angiosperms is homologous with the initial nucleus of the archegonium, which, as in *Gnetum*, matures without the formation of that organ; it therefore represents the latter.

'2. The synergidae probably represent the cells in the upper part of the prothallium, whose only function appears to be the nutritive one common to the whole, but which, as Strasburger suggests, have assumed in Angiosperms a new one in relation to the pollen-tube.

'3. The antipodal nuclei represent the nutritive and haustorial cells which compose the lower part of the prothallium.

'4. The upper polar nucleus represents the jacket-cells, which, though not as a rule capable of fertilization, yet, after union with another nucleus, appear to be in a condition to receive stimulus from the contents of the pollen-tube and to proceed to the formation of proembryos. Since in the Angiosperm embryo-sac there is reduction to one solitary nucleus, the stimulus from the pollen-tube naturally takes the form of fusion with the second male gamete' (11, pp. 283–4).

Certain details in the above scheme are perhaps open to criticism. The synergidae might well be considered to represent two initial cells, comparable with the archegonium initial represented by the ovum, and the jacket-cell initial represented by the upper polar nucleus. The lower polar nucleus is presumably of prothallial nature, like the antipodals, though this is not definitely stated. But these are mere details. Miss Berridge's comparison of the apogamous proembryos of *Ephedra* with the endosperm of Angiosperms throws a completely new light on this obscure subject, and suggests definite lines of research. Our knowledge of embryo-sac structure and of the details of fertilization in other species of *Ephedra* is so far very fragmentary. More complete information on these points, and on the corresponding features in *Welwitschia*, may possibly settle the vexed question of the homology of the endosperm, and at the same time determine the true position of the Gnetaceae in the Natural System.

Comparison with the embryo-sac of *Gnetum* is of the utmost interest, though at present it can be only tentative. We may suppose that the common ancestor of all branches of the Gnetaceae possessed an embryo-sac in which a group of archegonium initials occupied the apex, and true prothallial tissue the base. Some of these initials may have produced jacket-cells in the mature sac. Whether this were so or not, all the initials might be reduced alike to naked nuclei. Thus in *Gn. Gnemon* each of the equivalent nuclei in the apex of the sac, supposed by Dr. Lotsy to be all

equally capable of fertilization, would represent an archegonium initial in some ancestor, more or less remote. As Miss Berridge remarks, this hypothesis fully justifies Dr. Lotsy's assertion that the archegonium in *Gnetum* is reduced to a naked nucleus, and that reduction has in this respect proceeded further than in Angiosperms, where the ovum is a complete cell (55, p. 103).

At first sight the interruption of prothallial formation by the act of fertilization, as described by Karsten (50) in other species of *Gnetum*, recalls the Angiospermous history. But there is no real resemblance. When the pollen-tube enters the embryo-sac of *Gnetum*, the endosperm consists of numerous free nuclei embedded in its parietal cytoplasm. After the epoch of fertilization these nuclei appear to divide; cell-walls are formed round them; and development proceeds in the usual way until the embryo-sac is filled with tissue. The homology with the endosperm of other Gymnosperms would be clear even without the link supplied by Lotsy's description of *Gnetum Gnemon* (55). In the embryo-sac of Angiosperms, however, the whole endosperm begins with the mitosis of a fusion nucleus which contains a male element from the pollen-tube. Thus the endosperm is of necessity developed after fertilization, because the nucleus which gives rise to it is not complete until that epoch. No trace of such a process is found in *Gnetum*. The primary nucleus of the endosperm is not a fusion product. It has given rise to a number of equivalent nuclei before fertilization occurs, and growth is resumed afterwards by the simultaneous activity of these nuclei, or the greater part of them.

No tissue then in *Gnetum*, nor, so far as we know, in *Welwitschia*, can be considered as the direct representative of the Angiospermous endosperm.

To sum up, the germination of the embryo-sac and the history of the endosperm isolate Monocotyledons and Dicotyledons from all other plants. The only adequate explanation of the identity of two processes so complicated in two separate races is inheritance of these features from an ancestor common to both. The alternative explanation is independent evolution of both members along distinct lines of descent, and to attain identity in that way would require a series of coincidences so improbable as to be inconceivable.

The argument from these two features is very much stronger than the similar argument founded on the identity of the carpels in both classes, since the coincidences in structure are more numerous and more striking. The value of both arguments is much increased when they are considered together.

Monocotyledons and Dicotyledons have another member of great importance in common—their flower. But whether a morphologist considers

the possession of that member to be a character which isolates them from other groups will depend largely on the views he holds on the primitive form of flower.

The subject has been so fully discussed in Messrs. Arber and Parkin's recent paper (4) that I will not treat it at length here. The difficulty arises from the astonishing variety in the floral structure of the Angiosperms. No botanist has yet succeeded in framing a definition of a flower which will include every form among them and yet exclude the reproductive organs of all Gymnosperms and Pteridophytes. The presence of a carpel indeed separates hermaphrodite and female flowers in Angiosperms from the corresponding organs of Gymnosperms and other groups, but male Angiospermous flowers have also to be distinguished from the male cones of other plants. Nor does a definition depending on the presence of a carpel add any weight to the collective argument in favour of a monophyletic origin, since we have already considered that character separately.

Yet in practice it has been found the most convenient plan to confine the term 'flower' to the Angiosperms. Gymnosperms were long ranked among Flowering Plants, but the difficulty of describing their reproductive axes in floral terminology has produced quite a literature on the homologies of the Gymnospermous cone and its parts. This difficulty suggests that there is something unique about the flower of the Angiosperm. What then are its differentiating characters?

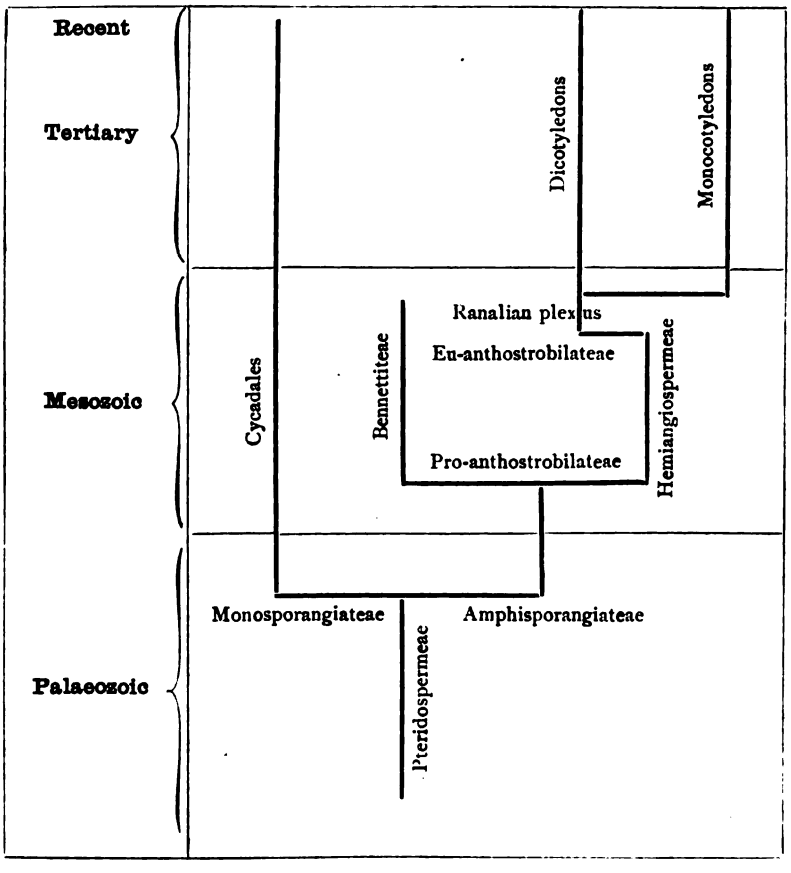
The complete flower with its concentric whorls of perianth-leaves, stamens, carpels, really is confined to the Angiosperms. Those botanists who maintain that all other floral forms are modifications of this type, reduced from it, are justified in declaring that a true flower is not found outside the group. On this view all Angiosperms display traces of a complete flower, and the presumption that it was inherited from a common ancestor is then very strong. The antiquity of the complete flower as compared with the simple unisexual type is very strongly supported by Messrs. Arber and Parkin. The subject will be better discussed when we attempt the reconstruction of the Primitive Angiosperm. For the present it is sufficient to say that I agree with this view, and the possession of a true flower is therefore in my opinion a further argument for the monophyletic origin of Angiosperms. But the case in favour of such an origin is already so strong that it is not necessary to insist on corroborative evidence.

Reconstruction of the Primitive Angiosperms.

In the previous section I have given reasons for believing that Monocotyledons and Dicotyledons are derived from a single stock which was Angiospermous in all its essential features. This race of plants may be called for convenience the Primitive Angiosperms. The term must be

understood to apply to the immediate ancestors only of our two groups of living Angiosperms.

By the kindness of the authors, I am able to reproduce here the Table of Relationships given by Messrs. Arber and Parkin on p. 77 of their memoir :—



In referring to this skeleton pedigree, I do not mean to attach more importance to the views it represents than do the authors themselves. They clearly regard it as a provisional statement, which will probably require amendment in the light of future research. But the merit of such graphic statements is to suggest that historical perspective which it is so difficult to preserve in dealing with phylogenetic problems.

In this Table the Primitive Angiosperms, defined as above, would stand in the main line of descent, just below the junction of Monocotyledons with Dicotyledons. We are entitled to assume that they possessed all those characters which are common to Monocotyledons and Dicotyledons. The

foliage of the leafy plant was Angiospermous: it bore flowers and seeds: its ovules were enclosed in carpels. The male gametophyte was represented by three nuclei within the pollen-tube. The female gametophyte before fertilization consisted of six cells and two free nuclei, orientated in a very characteristic way within the embryo-sac. The endosperm was formed after fertilization and arose from a fusion of three nuclei—two of them already free in the embryo-sac, and the third a generative nucleus from the fertilizing pollen-tube. All the nuclei of the endosperm must in consequence have possessed a number of chromosomes in excess of the sporophytic number.

These are for the most part very definite characters, but one of them opens out a question of great importance. What sort of flower was borne by the Primitive Angiosperm?

The classification of living Angiosperms is largely based on the external morphology of their flowers. The mass of observations on this subject accumulated by taxonomists represents the labour of many generations. As the natural system is now avowedly based on the principles of evolution, its divisions claim to represent degrees of kinship. The form of flower which the elder systematists would have called typical of an order, for example, is now understood to represent—at least in its main features—the flower of the ancestral stock from which all the genera included in that order are descended. The question of descent underlies all the problems of systematic botany.

Thus the evidence of taxonomists on the question of the primitive form of flower is by far the most weighty to which we can appeal. If they were substantially agreed on the form of flower which their comparative researches showed to be the most primitive, their collective authority would be overwhelming. But since they are divided, their conflicting opinions may be tested by those results of systematic botany which are the common property of all botanists, as well as by the knowledge derived from other sources.

Messrs. Arber and Parkin have undertaken this task (4). They agree with the elder systematists who considered the primitive flower as an elaborate structure, its numerous tepals, stamens, and carpels arranged spirally on the axis in acropetal succession. Such flowers occur in living species—for example in the *Magnolia* and other members of the Ranales. According to this view, all other floral types are derived by reduction from this.

The alternative view is held by most modern taxonomists, but not by all (Arber and Parkin, pp. 38, 39). It is agreed that a large number—perhaps the majority—of simple unisexual flowers are derived by reduction from more complex bisexual forms. But these complex floral types are supposed to be themselves built up from unisexual ancestors, and some

living forms which represent such ancestors are considered as the most primitive flowers known.

Three objections are raised against this view. 'In the first place it must be assumed that the perianth is evolved *de novo*, and is an organ *sui generis*. Secondly, in many of the groups regarded as primitive, e.g. Peperales, Amentiferae, and Pandanales, the inflorescence is a sharply defined and often a highly complicated structure. Lastly, such a theory has so far proved barren from a phylogenetic standpoint, especially when the attempt is made to bring into line evidence derived from the study of fossil plants' (Arber and Parkin, p. 39).

The first difficulty is obvious and hardly requires comment. The second must be attacked by taxonomic methods. The third is that which the authors develop at greatest length.

The substance of their argument is that the strobilus or cone is a very ancient type of fructification among Cryptogams and Gymnosperms, and that such a flower as that of the *Magnolia* can be interpreted as a bisexual strobilus in which the male sporophylls stand below the female, and both are protected by a special series of sterile leaves forming a basal perianth. Dr. Wieland (89) has recently shown that American members of the Bennettiteae possessed bisexual strobili of a form hitherto unknown. The male sporophylls stood below the female, and a number of sterile leaves below both. This form of strobilus may be fairly compared with an Angiospermous flower of the Ranal type. It is acknowledged that the individual Bennettitean sporophyll, male or female, differs very widely from the corresponding organ in Angiosperms. The points which the fructifications have in common are the arrangement of sporophylls of both sexes on a single axis, their relative position on that axis, and the presence of sterile members at its base.

In short, the description of the amphisporangiate strobili belonging to members of the Bennettiteae has suggested a possible origin for the Angiospermous flower (Wieland, 89, p. 245; Scott, 80, p. 139). There is nothing forced or unnatural about the scheme of development worked out in detail by Messrs. Arber and Parkin. But any such scheme requires that the more recent ancestors of our living Angiosperms should have borne flowers of the anthostrobiloid type, that is, flowers in which perianth leaves, stamens and carpels are arranged on the axis in acropetal succession. Such flowers are actually found among living Angiosperms. They have been considered primitive on taxonomic grounds by many authorities in the past, and are still thought to be so by some modern botanists. Their claims to antiquity are certainly much strengthened by the resemblance lately discovered between certain of their features and the corresponding features in extinct forms with Gymnospermous seeds.

It may perhaps be objected that the resemblance is superficial. Why

should so much stress be laid on the position of female above male sporophylls, and on the presence of sterile members at the base of the cone?

There is, I believe, no exception to the rule that in every hermaphrodite flower the carpels stand above the stamens. Because the arrangement is universal we are in danger of supposing it inevitable. But it would be difficult to find a physiological cause for this sequence which would apply to every case without exception. Indeed, in certain inflorescences which for all physiological purposes are equivalent to hermaphrodite flowers, the carpels stand below the stamens (*Arum*, &c.). This shows that such an arrangement may under certain circumstances benefit the plant.

In some heterosporous cones (e.g. *Lepidostrobus Hibbertianus*, quoted by Messrs. Arber and Parkin, 4, p. 38), the microsporophylls stand above the megasporophylls. The Angiospermous sequence is clearly not physiologically inevitable either in flowers or cones. The Bennettitean strobilus is said to stand alone among strobili in the sequence of its parts combined with the possession of a whorl of sterile members at its base (p. 37). This unique combination of characters finds a parallel in the Angiospermous flower.

The strobiloid theory of the flower seems in the present state of our knowledge to stand alone as a working hypothesis. If we reject it, we are left without any historical clue to the origin of the floral structure of Angiosperms. If we accept it, the Primitive Angiosperm must be credited with a flower resembling that of *Magnolia* or *Liriodendron* in general plan.

The Primitive Angiosperms have now been invested with certain characters which are common to Monocotyledons and Dicotyledons. Nobody who admits the existence of a single group of Primitive Angiosperms will question their right to these. The vexed question of their floral structure has been summed up in favour of the Ranal type on the grounds given by Messrs. Arber and Parkin. The characters in which Monocotyledons differ from Dicotyledons have not yet been discussed.

The two characters which separate Monocotyledons from Dicotyledons most completely are (1) the anatomy of the mature stem, and (2) the number of cotyledons. Less constant points of difference are found in the leaf and root structure, and in the floral symmetry.

The question before us is whether the Primitive Angiosperms resembled one class or the other in these respects, or were completely different from either. This third suggestion is unlikely; in all probability the Primitive Angiosperms resembled one branch of their descendants or the other in each of the characters in which those classes differ, but it is quite possible that they possessed some characters which we now call Monocotyledonous together with others which are proper to Dicotyledons. The evidence concerning each character must be considered on its own merits.

Before proceeding to the examination of this evidence, it should be mentioned that the controversy is a very old one. Botanists have discussed the comparative antiquity of Monocotyledons and Dicotyledons for several generations. For a long time the prevalent opinion was that Monocotyledons were older than Dicotyledons—in other words, that Primitive Angiosperms resembled modern Monocotyledons in the characters in which they differ from Dicotyledons.

Evidence in favour of this opinion was derived from three sources: the succession of fossil forms, the comparative anatomy of the stem, and the development of the embryo within the embryo-sac.

The fossil argument was founded on a mistake. It is now generally acknowledged that Monocotyledons do not appear before Dicotyledons. On the contrary, they are found together in the earliest beds in which Angiosperms appear at all (Seward, 80. *a*; Zeiller, 91, pp. 219–21). The arguments from stem anatomy and embryology will be considered under those heads.

Stem-structure.

As Primitive Angiosperms have not yet been identified in the fossil state, there is no direct evidence concerning their stem-structure.

The features common to the stems of Monocotyledons and Dicotyledons are three. Their vascular skeleton is built up in the first instance of leaf-traces only. Each leaf-trace while young is collateral in structure. The xylem of each leaf-trace is endarch. We may assume that the stem of Primitive Angiosperms possessed these characters.

The leaf-traces of the typical Dicotyledonous stem are arranged in a single circle. In each trace the xylem is internal and the phloem external, and they are divided from each other by a meristematic layer, the cambium. At first the cambium is not found outside the traces, but it soon forms a continuous cylinder within the stem, which produces secondary phloem on the outside and secondary xylem on the inside. The stem now in place of isolated leaf-traces displays a series of three concentric cylinders: centrifugal xylem, centripetal phloem, and meristematic cambium between them.

In the great majority of Monocotyledons no thickening-ring of any kind is formed. The numerous leaf-traces are scattered over the transverse section of the stem with apparent irregularity. Their origin from leaves with a more or less definite phyllotaxy leads to a primary arrangement in concentric circles, and each leaf-trace as it enters the stem is orientated with internal xylem and external phloem. At this level it is collateral in structure, as in the leaf itself. There is no layer of meristematic tissue within the bundle, consequently secondary elements are never added to it.

The leaf-traces of a Monocotyledon do not run parallel to the axis of the stem throughout their course, and the original arrangement in concentric circles is soon lost. Torsion of the trace on itself leads to irregular orientation, and even the collateral structure sometimes becomes concentric. The type of bundle called by Professor Jeffrey amphivasal (46, p. 315) is so common in the older stems that he figures it as the typical Monocotyledonous bundle (loc. cit., Fig. 113, *H*,*H*). In this form of concentric bundle the phloem is completely surrounded by xylem. Professor Quéva points out that in the stem of *Gloriosa* concentric bundles are due to anastomoses of collateral traces with each other (65, p. 87 and Figs. 125, 126). This is certainly the case in the scutellum of *Zea Maïs* (Sargant and Robertson, 74, p. 121 and Figs. 15–18). In the lower part, where branches are absent, the main bundle is collateral. But in the upper part, where branches are inserted all round the main bundle, it becomes almost amphivasal.

Thickening-rings are formed in the cortical parenchyma of certain Monocotyledonous trees, but they do not constitute a cambium in the Dicotyledonous sense.

The numerous differences in detail which separate the stem-structure of Monocotyledons from that of Dicotyledons depend more or less directly on the presence or absence of an active cambium. Its presence secures a single ring of leaf-traces, the persistence of their collateral structure, and their uniform orientation. This is illustrated by the structure of Dicotyledons in which the cambium has become inactive and is disappearing. In such forms we find not only the scattered arrangement of the traces (Holm on *Podophyllum*, 45, pp. 429–30. Cf. also Fig. 10 on p. 52 of Solereder, 81), but even occasional amphivasal structure (Worsdell, 90, p. 600). It was a true instinct which led early botanists to lay so much stress on the presence of a cambium. The character it affords is also one of the most satisfactory to taxonomists, for, though some Dicotyledons have lost the cambium, it is not found as a really active tissue in any Monocotyledonous stem.

The question really is, then, whether the Primitive Angiosperms possessed a cambium. Evidence of two kinds is available: comparison of the Angiospermous stem with that of Gymnosperms and Cryptogams, and comparison of the mature stem of Monocotyledons and Dicotyledons respectively with the seedling stem of the same class.

A cambium is the rule among living Gymnosperms, and universal among the extinct forms yet investigated. Traces of its existence are very rarely found among living Vascular Cryptogams (e. g., see Cormack, 17). The absence of a cambium was formerly considered as a conclusive mark

of the low place in the botanical scale held by Vascular Cryptogams. For many years fossil botanists were accustomed to place all the stems with normal secondary thickening among Phanerogams without further question. While this view prevailed, the absence of cambium in the stems of Monocotyledons was naturally considered as a primitive character. Now, however, a cambium is known to be the rule among the extinct Vascular Cryptogams whose tissues have been sufficiently well preserved for microscopic examination. The marked difference between living and extinct forms in this respect is no doubt due to the fact that living Vascular Cryptogams are herbaceous, while the forms preserved as fossils are mainly trees. In such forms a cambium would be found if it existed within the group at all.

The evidence then shows that a cambium is much more ancient than the Primitive Angiosperms, and that they probably inherited one, from whatever race of Gymnosperms or Vascular Cryptogams they descended.

Comparison of seedling with mature Dicotyledons shows that the ring of leaf-traces is visible in the plumule so soon as vascular tissue is differentiated within it. Cambium is present from the first within each trace, and appears between them very shortly. The single ring of open bundles is formed even in the seedlings of such forms as *Podophyllum*, in which the later bundles are closed and scattered within the stem.

Thus the primary stem-structure of Dicotyledons is the same in the seedling as in the mature plant. If the Monocotyledonous type were primitive, we might expect some of its features to appear in the young Dicotyledon. Amphivasal closed bundles should be formed first, and pass gradually into the open collateral form. But this does not happen. The earliest bundles formed are collateral, and have a well-marked cambium.

Among Monocotyledons, on the other hand, the characteristic stem-structure is reached only when the stem approaches maturity. The bundles of the seedling internodes are collateral, and frequently contain traces of a cambium. Their arrangement within the stem can be traced satisfactorily only in the rather exceptional forms in which the early internodes are well developed. In such forms Professor Jeffrey (46, pp. 314, 315) has pointed out that the bundles of the first internodes are commonly arranged in a single circle, and orientated as in Dicotyledons. It is true that, while there are still very few leaves, their traces would naturally fall into a single circle. But their regular orientation in that circle—xylem inwards, phloem outwards—suggests that they were formerly linked together by a cambium. Besides, the occasional presence of a short-lived cambium within the traces of the seedling, and their strictly collateral structure, are very strong evidence for supposing that to be the primitive structure of the leaf-trace instead

of the closed type, which is universal in the mature Monocotyledon, or the amphivasal form which is characteristic of it.

Traces of a cambium in the vascular bundles of Monocotyledonous seedlings have been recorded by several observers. Miss Anderssohn (1) in 1887 figured a cambium within the bundles of seedlings belonging to thirteen species. The tissue is clear in all her figures: it would seem best marked in *Zea*, *Typha*, *Lilium*, and *Dracaena*. Among my own preparations there are ten well-marked instances from eight genera. I found the greatest development of cambium in the hypocotyl of *Yucca arborescens*. It is quite clear in the same region of two other species in that genus, *Y. gloriosa* and *Y. aloifolia*. Cambium appears also in cotyledonary bundles of *Milla*, *Dipcadi*, *Galtonia*, *Albuca*, and *Fritillaria*. *Elettaria* and *Musa* show it in the traces of the first and second leaves.

The cambium in all these forms is very transient. It is not seen in the very young bundle, where the protoxylem is only just lignified. Such a bundle is often wedge-shaped in transverse section. The lignified protoxylem is at the sharp angle of the wedge, a group of sclerenchyma at the broad end, and just within the sclerenchyma a few elements of 'soft bast'. Between protoxylem and protophloem is an ill-defined region, of which the elements bordering on the protoxylem will become metaxylem, and those adjacent to the protophloem will form more phloem. Before this process is complete, three or more rows of cells between phloem and xylem will often be found in radial series. The innermost rows will be added to the metaxylem, the outermost to the phloem. Divisions in this region soon cease, and alterations of shape and size—perhaps a few irregular divisions too—in the elements added to the bundle usually destroy the original radial sequence. When this has disappeared, no trace is left of the formation of cambium.

Professor Quéva has worked out the anatomy of *Gloriosa superba*, a tropical Liliaceous climber, from germination to maturity (65). The plant produces fresh aerial stems every year. They die down at the end of each growing season. The perennial organ is a V-shaped tuber, which is simply a branched and thickened segment of the stem. The tuber lasts through two seasons. In the first it is formed on the parent tuber and grows to its full size. In the second it renews its growth, giving rise to two aerial stems and two new tubers. This activity exhausts the food laid up in the first year, and brings about the death of the tuber.

The bundles of the seedling stem possess a short-lived cambium, which adds phloem and xylem elements to the complete structure (Quéva, p. 102). Traces of its activity are soon obliterated by subsequent divisions in the secondary elements, and by irregularities of growth which destroy their original arrangement in rows. The bundles of the stem-segment which becomes the tuber of the seedling plant have lost all traces of

this early cambium, and secondary growth is never resumed in this tuber.

The bundles of the second tuber, however—that which springs from the tuber of the seedling—possess cambium which is particularly active in the second season. Professor Quéva figures the radial rows of elements added to phloem and xylem (65, Figs. 72 and 90). He does not describe the formation of intrafascicular cambium at any time.

All succeeding tubers show a similar formation of secondary tissue, more massive than in the second tuber (p. 56, and p. 58).

The presence of a functional cambium in the mature bundles of a Monocotyledon may be advanced to show that they are derived from an ancestor which possessed one. Professor Quéva takes this view:—

'The persistence of a cambial zone in the bundles of certain Monocotyledons shows that we may logically consider them as derived from the more primitive Dicotyledons by means of the early disappearance of the cambium, and an increase in the number of traces from each leaf.' (Translated from 65, p. 147.)

Comparison of seedling anatomy in the two classes, then, leads to the conclusion that the dicotyledonous stem-structure is primitive, the monocotyledonous derived from it. This conclusion agrees with that founded on comparison of the mature stem anatomy in both classes with that of other groups, living and extinct. The presence of a cambium is the rule, its absence the exception. Except in *Gloriosa*, it is completely lost in the mature stem of Monocotyledons, but it can still be found occasionally in the traces of their seedlings.

The Primitive Angiosperms, then, must be credited with a cambium, which has been very completely lost by one branch of their descendants.

Number of Cotyledons.

Monocotyledons differ from Dicotyledons in other mature characters besides the structure of their stems. But these are far less constant, and may be considered later. An embryonic character—the difference in the number of cotyledons—must come first, for in systematic importance it ranks with the stem anatomy, or even takes place of it.

No Monocotyledon with which I am acquainted has two cotyledons. Some Dicotyledons, indeed, have only one. The species with this abnormal feature are widely separated in the Natural System. They belong to the Ranunculaceae, Fumariaceae, Umbelliferae, Primulaceae, Lentibulariaceae, Nyctaginaceae (72, p. 76). Perhaps the species of *Peperomia* lately described by Mr. A. W. Hill also belongs to this list (39). Altogether I cannot find quite twenty species of Dicotyledons in which this character has been recorded. The possession of one cotyledon then is universal among Monocotyledons, and the possession of two very nearly so among Dicotyledons.

The question is whether Primitive Angiosperms had one cotyledon or two. Evidence of two kinds is available. Living Angiosperms may be compared with other groups. If the number of cotyledons found among lower plants, living or extinct, be at all constant, there would be some reason to suppose that number to be derived by Primitive Angiosperms from their ancestors.

In the second place, there is embryological evidence. The development of the embryo in both classes may perhaps repeat its race-history in some degree. Many observations have been made on the development of the embryo within the embryo-sac, that is, on the period in the history of the young plant which begins with the division of the fertilized ovum and ends with the formation of the ripe seed. The next period commonly begins with germination. The embryo of the ripe seed has merely to elongate its members and push them out of the seed-coats in order to become the seedling.

But this is not always so. In a considerable number of species belonging to both classes, but particularly numerous among Monocotyledons, the embryo passes through a period of maturation between the ripening of the seed and its germination. In such species the embryo is not ready for germination by the time the seed is ripe; it resumes its growth within the embryo-sac when sown under proper conditions. Seeds containing such embryos lie dormant in the soil for months after they have ripened and been shed from the plant. Germination is of necessity postponed until the embryo is fully differentiated.

For our present purpose, however, the epoch of germination is the most convenient division. The embryological evidence will be considered under two heads: the development of the embryo within the embryo-sac, whether that development is complete within the ripe seed or not, and the development of the seedling after germination.

Let us begin by comparing Angiospermous cotyledons with those of other plants. The Gymnosperms alone possess cotyledons which are clearly equivalent to them. Among Gymnosperms two cotyledons are found in the *Cycads*, in the Gnetaceae, in the extinct Bennettiteae, in *Gingko*, the Cupressineae, and the Taxaceae. More than two are found among most Abietineae and Taxodineae. The Araucarieae differ. *Agathis* has two cotyledons, *Araucaria* two to four.

Two cotyledons, then, are very frequently found among Gymnosperms. One is unknown, unless it occur in *Ceratozamia*.[1] The more primitive forms, such as the *Cycads* and *Ginkgo*, have two. So have the Gnetaceae and Bennettiteae, two groups which in other characters approach Angiosperms. With the exception of the poly-cotyledonous *Araucarias*, those groups which

[1] Eichler (22), p. 17.

possess more than two cotyledons have little claim to be regarded as primitive on other grounds. Moreover, there is some reason to think that where more than two cotyledons are present they have been formed by the splitting of an original pair (Hill and de Fraine, 42; p. 473).

Comparison with Gymnosperms is thus—so far as it goes—in favour of the assumption that two cotyledons were transmitted to Primitive Angiosperms by their ancestors.

Embryology within the Embryo-sac.

The embryological evidence is divided, as already explained, under two heads. We will begin with the development of the embryo within the embryo-sac.

The development of *Capsella* among Dicotyledons, and of *Alisma* among Monocotyledons, is described and figured in all textbooks. The figures are commonly reproduced from Hanstein's memoir of 1870 (35). Hanstein himself described several variants on the course of events which he considered typical, and later research has brought others to light. The great majority of Dicotyledons hitherto investigated do not, however, differ much from *Capsella*. More variation seems to occur among Monocotyledons.

In *Alisma* and *Capsella* the cotyledons are the first permanent organs formed in the pro-embryo. The growing-points of root and stem appear later. This is the regular order of development in Monocotyledons and Dicotyledons alike. Leaving such abnormal forms as *Cuscuta* (Koch, 51) out of the question, the only exceptions with which I am acquainted are found among the Papaveraceae. In *Roemeria refracta*, figured by Hanstein (35, Pl. VII, Figs. 6 and 8), the growing-point of the stem is indicated almost as soon as the rudiments of the cotyledons. Hegelmaier has shown that in *Hypecoum procumbens* the stem-apex appears at the same time as the cotyledons, or even a little before them (37, Pl. III, Figs. 23-6).

The free end of the embryo increases in size in both types. In *Capsella* it bifurcates to form the two cotyledons: in *Alisma* the whole is transformed into a single cotyledon. The growing-point of the root appears at the other end of the embryo, separating it from the suspensor. It is alike in both types. The growing-point of the stem appears between the cotyledons in *Capsella*, but at one side of the pro-embryo in *Alisma*—just below the single cotyledon.

To avoid error in the interpretation of these results, the goal must be constantly kept in view. The question before us is whether the Primitive Angiosperms possessed one cotyledon or two. Can the facts just rehearsed be used as evidence on either side?

The simplest interpretation is certainly that the single rudiment found in both classes remains undivided in Monocotyledons, but divides in

Dicotyledons. This hypothesis was readily accepted by observers already convinced on independent grounds that Monocotyledons were the elder race. Now that these grounds are given up, the embryological evidence stands alone and must be criticized on its own merits.

The chief difficulty in the way of accepting this solution is that the cotyledon of *Alisma*, for example, is to all appearance a terminal structure. If this represents the ancestral form, the two cotyledons of *Capsella* must be considered as due to fission of a terminal member. But the typical leaf is formed laterally on the growing-point of an axis. The rest of the growing-point forms other lateral members, at the same time reproducing itself. Can a member formed from the whole of the growing-point—that is, terminally to an axis which then ceases to form new members—be a true leaf?

Many morphologists think that it cannot. If we consider the pro-embryo to possess an axis, there is no denying that the single rudimentary cotyledon of Monocotyledons, or the bifid rudiment which represents the pair in Dicotyledons, is for a time apparently terminal. Hence some botanists maintain that the cotyledons cannot be considered as true leaves.

Those who hold this opinion belong to two schools. The first draws a distinction between Monocotyledons and Dicotyledons. The cotyledons of the latter may be regarded as lateral members. The apparently terminal position of the rudiment from which they are derived is due simply to the arrest of the growing-point. Though commonly formed later than the cotyledons, it is terminal from the first. Forms such as *Hypecoum*, in which the growing-point appears at the same time as the cotyledons, or earlier, represent the primitive arrangement. The single cotyledon of Monocotyledons is, however, truly terminal, and cannot be treated as homologous with one or both cotyledons in Dicotyledons. It is an organ *sui generis*, and can never under any circumstances be considered as a leaf. Its leaf-like appearance in some seedlings is due to homoplastic adaptation (Balfour, 8, pp. 827–8; cf. also Coulter and Chamberlain, 19, p. 208).

We need not consider this hypothesis at greater length, since it is hardly compatible with the monophyletic origin of Angiosperms. Indeed its supporters commonly assume a polyphyletic origin.

The second school is represented among recent writers by Professor H. L. Lyon (57, 58). He considers the single cotyledon of Monocotyledons as truly terminal, and both cotyledons of Dicotyledons as derived from it by fission. This hypothesis he has carried out to its logical consequences. His views are founded on the structure of the embryo in *Nelumbium*. He has shown that in *N. luteum* the embryo has no suspensor, and becomes a large, undifferentiated, nearly spherical mass before the cotyledons are indicated. They appear as a crescent-shaped ridge, which

soon becomes bi-lobed, and then gives rise to a pair of very long cotyledons (56).

Here there are three variations on the *Capsella* scheme: the absence of a suspensor, the size of the undifferentiated embryo, and the first appearance of the cotyledons as a single crescent rather than as two distinct rudiments. Of these anomalies, the last is the most important. Many instances are recorded of embryos without a suspensor. It is not very uncommon to find an embryo which attains considerable size before the cotyledons are indicated. Hegelmaier figures an undifferentiated embryo of unusual size in *Helleborus foetidus* (37, Pl. II, Fig. 19), and Johnson describes a similar case in *Heckeria* (48, pp. 328–9 and Fig. 29).

Professor Lyon considers that in *Nelumbium* we can trace the development of two cotyledons from a single terminal member, which is derived from a similar member in the embryo of the Primitive Angiosperms and is homologous with the single cotyledon of Monocotyledons. He faces all the consequences of this hypothesis. The ancestral member, being terminal, is not a phyllome but an organ *sui generis*—perhaps derived from the foot of Vascular Cryptogams, and homologous with the sucker in seedlings of *Gnetum* and *Welwitschia* (Bower, 13, 14). The derivatives of such a structure have, of course, no claim to be considered as true leaves. Hence the leaf-like structure of the cotyledons in most Dicotyledons and some Monocotyledons must on this view be attributed to their adaptation to the functions of leaves.

Comparison of the anatomical structure of green cotyledons with that of true leaves shows a remarkable resemblance between them. Professor Ramaley's descriptions, for example (66, 67), show identical structure in epidermis, stomata, palisade and spongy tissue, and in the details of the vascular bundle. The differences on which he insists appear to me of minor importance. There are differences in the distribution of stomata, in the number of rows of palisade cells, in the distribution of the bundles.

Schlickum (77), after careful comparison of the cotyledon with the first leaf in a number of Monocotyledons, came to the conclusion that they were homologous with each other. I am confirmed in the same opinion by examination of a series of preparations most kindly made for me by my friend Miss Berridge. She has mounted sections from the cotyledon and first leaf of various Dicotyledons, side by side, and the resemblance in structure is very clear.

In short, to deny that cotyledons are homologous with leaves introduces new difficulties into a subject already sufficiently obscure. Such difficulties are the logical consequences of the three assumptions on which Professor Lyon's theory is based: that the single cotyledon of Monocotyledons is terminal, that a terminal member cannot be a leaf, and that both cotyledons

of the Dicotyledonous embryo are derived from the single terminal cotyledon of the Primitive Angiosperms by fission.

Two ways of escape are open. We may accept the possibility of a terminal leaf, or we may try to show that the apparently terminal cotyledon is derived from a lateral member.

To begin with the question whether a leaf can be truly terminal. Some botanists attempt to solve the problem by saying that the cotyledon is an active organ of the embryo and assumes the position best suited to its work. That statement is no doubt very true, but it leaves the morphological question untouched. When a member assumes new functions, its structure is of course modified in the course of generations to suit those functions. But modification of a member already fairly well developed rarely goes so far that no trace of its original structure remains either in the embryonic or the mature condition. If such complete metamorphosis were usual, there would be no science of comparative morphology at all.

When a morphologist says that a leaf is essentially a lateral member, he is stating a wide generalization in the form of a law. Experience shows that leaves and leaf-like members are arranged laterally on an axis, which increases in length by means of a terminal growing-point. The rudimentary leaf appears as a lateral out-growth of this terminal meristem.

This statement is undoubtedly justified as a generalization, for instances of leaf-like members not clearly lateral in formation are very rare. Where an axis is present and the whole of its terminal growing-point is converted into a single member, there is a very strong presumption against that member as a leaf. The burden of proof rests with those who assert it to be so.

The cotyledon of *Alisma*, however, hardly comes under this rule. As Hanstein pointed out in 1870, neither axis nor growing-point is present when it is formed (35, p. 40, pp. 58–61, pp. 90–98). The pro-embryo is meristematic throughout, and is undifferentiated except for the external distinction between suspensor and embryo proper. The direction of the future axis is first indicated by the formation of a plerome cylinder. As a rule, no specialized growing-point is formed for some time afterwards.

The independent results of Hegelmaier (36) and Fleischer (25) showed in 1874 that the projection of tissue called by Hanstein the stem-apex is in some Monocotyledons the first leaf. The second is developed directly from a region near the base of the first, the third from the second, and it is sometimes a long time before a rudiment appears which becomes the apex of a true axis. In fact, for some time in such an embryo each successive leaf is terminal in the same sense as the cotyledon. When the rudimentary axis of such an embryo does appear, it is apparently a lateral outgrowth from the base of the last formed leaf. By degrees, as the axis becomes better defined, its growing-point assumes a terminal position, and the succeeding

leaves appear lateral. Are we therefore to conclude that the first two or three leaves in *Sparganium* (36, pp. 648–52) and the first seven or eight in *Pistia* (56, pp. 692–3) are really cauline structures which have become undistinguishable from true leaves in response to the demands of the environment, while the succeeding leaves alone have a morphological right to the name?

Those who shrink from this conclusion are faced by two alternatives. They must suppose either that the leaves are only apparently terminal through suppression of the axis, or—with Celakovsky (16)—that leaves were originally terminal members, that the stem began as a symposium of leaf-bases, and that this primitive construction survives only in the embryo and young seedling of a few Monocotyledons. The gradual transition from the terminal to the lateral position, which can be traced in the successive leaves of such forms, would then reproduce a similar transition in the history of the race.

The species which on this view are most primitive among living Angiosperms are, of course, those in which most terminal leaves are formed before the appearance of an axis. In Hegelmaier's researches *Sparganium* was found to have two or three terminal leaves, *Pistia* seven or eight. Both are aquatic forms. *Pistia* is very highly specialized to that habit. The seed before germination remains under water, but soon after germination begins it floats up to the surface, and further development proceeds there. The embryo of *Pistia* is much reduced: neither a suspensor nor a primary root is formed.

Fleischer's best examples are *Juncus glaucus*, a semi-aquatic form, and *Luzula multiflora*, an Alpine species.

My own experience is that the reduction in structure so characteristic of aquatic species is very strongly marked in their young seedlings. For this reason I have rarely found such seedlings of much use in my own work. Ancestral features have commonly disappeared or become obscure in the general loss of differentiation. Thus the fact that terminal leaves seem characteristic of aquatic embryos suggests very strongly that their peculiar position is due rather to suppression of the axis than to any reminiscence of a stemless period in the history of the race.

If we adopt this view, the complete suppression of the axis in the embryo and seedling of *Pistia*, for example, must be due to adaptation of the embryo itself to its surroundings. If the absence of an axis were a mature character which had become embryonic in the course of evolution, we should expect traces of a stem in the embryo which would disappear in the mature plant. But the converse is the case. The stem exists in the mature plant, and is absent in the embryo and seedling.

In the case of *Pistia* the conditions of germination are peculiar, and will account for very great adaptations in seedling structure. But less is

known of the conditions under which the seeds of *Sparganium*, *Juncus*, and *Luzula* germinate, and therefore no precise estimate can be given of the extent to which they might affect the structure of the embryo. All that can be said is that considerable reduction of structure commonly occurs in the embryo and seedling of aquatic and semi-aquatic plants.

The stem-axis of bulbous Monocotyledons is often all but suppressed in the embryo and seedling. Fleischer figures and describes the embryo of *Leucojum aestivum*. When two leaves are present within the cotyledonary sheath, the stem-apex which was between them is represented only by a limited region of actual cell-division. My own preparations furnish many instances of extreme suppression of the stem in the seedlings of leafy bulbs—*Allium*, *Fritillaria*, *Galtonia*, for example. In such seedlings the stem is often more reduced as compared with the leaves than it is in the mature plant.

The fact that reduction has proceeded further in the seedling than in the mature plant admits of two explanations. It may be said that the absence of stem is a primitive feature, and therefore better marked in the young plant, according to the law of recapitulation. If this were the true explanation we should expect reduction of this sort to be most marked in species which on other grounds appear primitive. But this is not so. It is most clearly seen in aquatic forms and in Alpine or bulbous forms—all highly specialized types.

If this explanation be rejected, the alternative is to suppose that in these exceptional forms the causes which result in suppression of the stem-axis operate more powerfully on the seedling than on the mature plant. In many of the instances quoted, this can be shown to be true. The seeds of aquatics, for example, commonly germinate under water, and thus the seedling is completely submerged, to begin with. It leads a more completely aquatic life in many cases than the adult plant which is only partially submerged (*Sparganium*). Extreme reduction in all non-essential parts is essential in the seedling of *Pistia*, which has to become light enough to float up to the surface.

The reduction of the seedling in Alpine and bulbous plants is due to other causes. For a full discussion of these I must refer to previous papers (Sargant, 72, pp. 78–81, and 73, pp. 334–5). The essential point is that a seedling germinating in the short summer of an Alpine summit is faced with a task almost beyond its powers. If it is to survive the coming winter, the seedling must be safely buried in the ground by the end of the summer, and be provided also with a reserve-fund of food. The stem of such a seedling is commonly developed as a tuber in which food is stored. It rarely develops internodes in the first years of its life. Bulbous plants bear traces of evolution under conditions equally stringent, though many of them have to resist hot drought rather than cold in the dead season.

More time has been spent on the structure of Monocotyledons in which the early leaves appear terminal than such exceptional forms would deserve, were it not for the light which their structure throws on the normal formation of an apparently terminal cotyledon. If there is reason to think that the influence of environment can effect a change in the configuration of the embryo and seedling so complete that the early leaves appear terminal owing to suppression of the stem, equally stringent conditions may account for the formation of an apparently terminal cotyledon.

What are the conditions under which the embryo is formed within the embryo-sac? In general it may be considered as a mass of meristem developing in a confined space, feeding parasitically on the tissues of the mother-plant, and obliged to prepare for a period of rest followed by one of rapid growth and severe competition.

The more obvious conditions which will affect the form of the embryo while it is developing within the embryo-sac are:

1. The space at its disposal, depending partly on the shape of the embryo-sac, and partly on the development and texture of the endosperm.

2. The method of food-supply.

3. The configuration of the mature embryo—that is, of the embryo immediately before its exit from the seed. This again depends on two sets of conditions: the future form of the seedling, which is largely determined by its environment after germination, and the method by which the embryo will free itself from the tissues of the seed.

We have very little exact knowledge on any of these points; the following remarks on each are merely suggestive.

1. The embryo-sac in the ripe seed of many species—Monocotyledons and Dicotyledons alike—is very often long, narrow, and sharply bent on itself. Mrs. Schaffner's drawing of *Capsella* (76, Fig. 37), Miss Gibbs' drawing of *Stellaria* (28, Fig. 22), and Professor Schaffner's drawing of *Sagittaria* (75, Fig. 73), show how the embryo has to accommodate itself in each case to its quarters. The embryo-sac continues to increase in size during the growth of the embryo, which in such forms is confined from the first within a long narrow space. It is further hampered by the presence of a growing endosperm.

2. The food-supply of the pro-embryo is no doubt commonly conveyed through the suspensor. In some forms—as in many of the Leguminous species described by Guignard (31), and those from the Rubiaceae described by Lloyd (54)—the suspensor is very highly specialized as a haustorial organ, is massive and persistent. But, as a rule, it flourishes for a limited period only; long before the seed is ripe the suspensor is withered and clearly no longer functional. Food must then reach the embryo in some other way.

The cotyledons are generally understood to take over the absorptive

function, and no doubt this is very often true. The few careful observations on the method of food-supply to the growing embryo indicate, however, that there is far more variation in the process than was commonly supposed. Two examples illustrate this.

Professor D. S. Johnson (48) gives good reason to think that in *Peperomia* the food is supplied to the growing embryo, as to the young seedlings, through the cotyledons. These organs have merely, however, to absorb the food in a soluble form. The work of drawing on the reserves laid up in the perisperm, and preparing them for use, is performed by the scanty endosperm.

Similar results are recorded by Miss Gibbs (28) for the Alsinoideae, with one important variation. Here, as in *Peperomia*, the endosperm prepares the food which it draws from the perisperm for use by the embryo. But in the Alsinoideae—in *Stellaria media*, for example—the suspensor is for a long time the absorbent and digestive organ. When the digestive function is assumed by the endosperm, that of absorption falls to the root-tip.

Much more work is needed on this question before we can hope to understand the morphology of the embryo. If the cotyledons commonly absorb food from the endosperm and pass it on, their early appearance in the history of the embryo is sufficiently explained.

3. Finally, the development of the embryo is modified by conditions other than those which act on it directly. The embryo of the ripe seed is not always mature. But whenever the embryo becomes mature it shows signs of adaptation to its past and present environment, to the environment of its immediate future, and to that of a future rather more remote. We have already touched on its adaptations to the conditions surrounding it during the period of growth which has just ended. The next stage will be the exit of the embryo from the seed, and certain features in the mature embryo can be referred to the difficulty of getting free quickly and without damage from the tissues which envelop it in the seed. The peg of gourd seeds is a well-known example (21, p. 22 and Fig. 5), and the action of the wedging roots in the Maize (74, p. 116 and Figs. 6–8). Finally, the members of the young seedling are present in the mature embryo, and their structure even in the undeveloped state has a certain reference to the conditions which will be encountered by the seedling. The coleoptile of Grasses, for example, is clearly adapted to pushing up through the soil.

The conditions, then, to which the embryo must conform are sufficiently complicated to account for wide departures in structure from the ancestral type. The question before us is whether those conditions would be likely to produce temporary suppression of the axis in a Monocotyledonous embryo, leading to the formation of an apparently terminal cotyledon. This question cannot as yet be answered directly, for lack of evidence. It is hopeless to attempt to appreciate the effect of conditions so imper-

fectly understood. But comparison of the normal type with certain exceptional forms is very suggestive.

Solms-Laubach (82) has described the development of the embryo in certain genera belonging to the Commelinaceae and Dioscoreaceae. He has shown that in such forms the cotyledon is lateral from the first, while the stem-bud is terminal. In his figures of *Tamus communis* the development of the cotyledon is traced from the beginning. It appears as a circular ridge surrounding the region in which the stem-bud will afterwards be formed. Growth in one part of the ridge soon ceases; the opposite region grows rapidly, and the rudimentary cotyledon finally arches over the hollow which contains the stem-bud. In the end the mature embryo is much shorter and broader than that of *Alisma*, but does not differ very widely from it in general shape.

The embryo-sac is not included in these drawings, but figures in Le Maout and Decaisne's textbook (52, p. 795) show the berry and ripe seed whole and in section, as well as the seedling during germination. The ovules are anatropous; the embryo-sac therefore is not bent upon itself as in *Alisma* and *Capsella*. The seed is very little longer than broad, and the embryo has in consequence plenty of room to develop laterally during the process of maturation. In Commelinaceae the ovules are peltate (52, p. 868), and of course much broader than long. Here again the embryo has had ample elbow-room within the embryo-sac.

This association of a lateral cotyledon with an unusually wide embryo-sac is very suggestive. Perhaps the apparently terminal position of the cotyledon in *Alisma* and the majority of Monocotyledons may have something to do with the long narrow embryo-sacs which are so common. The complete explanation would probably depend on other conditions too. We know far too little of the environment and needs of the growing embryo to attempt such an explanation yet.

Three hypotheses to account for the terminal position of the cotyledon in *Alisma* and most Monocotyledons have now been discussed. First, that it is a terminal member and therefore no true leaf. Secondly, that it is a terminal member and one of the few leaves surviving to represent the original or ancestral leaf, which according to this view was essentially terminal. Thirdly, that it is a lateral member, forced into a terminal position by causes which we do not fully understand, but believe to be adequate to the task.

Of these three suggestions the last seems the most satisfactory. If adopted, what bearing has it on the original question—the number of cotyledons possessed by the Primitive Angiosperms?

Suppose the single cotyledon of the ancestral Monocotyledon was lateral, was it derived from a single lateral cotyledon in the Primitive

Angiosperm? If so, the Dicotyledonous pair must have been formed either by the fission of a single lateral member, or by the addition of a second cotyledon to that already in place. The second suggestion is clearly improbable: the first deserves consideration. Such a cotyledon as that of *Tamus* might conceivably divide, and the segments shift their position until they faced each other. The embryo of *Nelumbium* as described by Professor Lyon might represent a stage in this process, though—as we have seen—this is not his own interpretation of the facts.

If, on the other hand, the Primitive Angiosperms had two cotyledons, and we treat the single cotyledon of Monocotyledons as a lateral member, it might be derived from the ancestral pair either by suppression of one of them or by the complete union of both.

The embryology of the Pseudomonocotyledons is of the greatest interest in relation to this subject. Scattered here and there within Dicotyledonous genera are a few species which have but one cotyledon. Hegelmaier (37) followed the development of the embryo in three of these species from the fertilized ovum to the ripe seed. They are *Ranunculus Ficaria*, *Corydalis cava*, *Carum Bulbocastanum*.

Unfortunately the embryo of all three species is very little developed in the ripe seed. A long period of maturation is necessary before germination can take place. Hegelmaier failed to secure the maturation stages, and his work was first completed in 1902 by the publication of a posthumous paper by Schmid (78), which carried the history of the embryos on to the epoch of germination. About the same time Sterckx (83) published a complete account of the development of the embryo in *Ranunculus Ficaria*.

In each of these species the single cotyledon was certainly derived from two. They belong to dicotylar genera; in other words, the ancestral *Corydalis*, *Carum*, and *Ranunculus* possessed two cotyledons. The single cotyledon in the aberrant species is an adaptation peculiar to themselves. Thus the change from a dicotylar to a monocotylar form has occurred in comparatively recent times—at any rate since the date of the original representative of each genus. Here, if anywhere, we might hope to trace the development of so well-marked a character in the young embryo. We should perhaps expect to find the rudiments of two cotyledons at first, then to trace either the development of the one and the arrest of the other, or their fusion into a single member.

The actual process in all three forms bears a strong resemblance to the development of the embryo in *Tamus*. The cotyledon appears on the flattened apex of the pro-embryo as a peripheral ridge. At first it is circular, but it soon becomes crescent-shaped by the rapid growth of one side. The stem-apex is often late in appearing. It is always formed in the central depression outlined by the circular ring. Very soon the

stem-bud is completely dominated by the lateral member, which embraces and at last arches over it. This lateral member is of course the single cotyledon, which becomes apparently terminal in the mature embryo, for it manages to push the stem-apex to one side.

In the interpretation of their results, Hegelmaier and Schmid consider one possibility only—the formation of a single cotyledon from the original pair by suppression of the second. Hegelmaier is inclined to localize the missing cotyledon in that region of the ring where growth first ceases. A projection is formed there from which a sheath-like organ is developed. Schmid, however, considers the projection in this place as equally likely to represent the sheath of the single cotyledon only, and he does not attach much importance to the single abnormal embryo of *Carum Bulbocastanum* figured and described by Hegelmaier in which this projection is almost as large as the still rudimentary cotyledon (37, Pl. VII, Fig. 41). Solms-Laubach definitely refused to consider a similar formation in the embryo of *Tamus communis* as anything more than the rudiment of the cotyledonary sheath (82, pp. 85, 86, and Figs. 29–33).

Sterckx (83), on the other hand, dealing with *Ranunculus Ficaria* only, is much influenced by the bilobed blade of the fully expanded cotyledon (p. 45 and Figs. 176, 177, Pl. XV). The peculiar venation of this blade suggests—as he points out—the fusion of two leaves into a single member. The embryo in his figures also shows a slight but quite definitely bilobed structure (loc. cit., Fig. 156). He concludes that the single cotyledonary member is in fact a fusion of the two cotyledons which we attribute to the ancestral *Ranunculus*.

Now, if we agree in this explanation of the single cotyledon of *Ranunculus Ficaria*, we may equally well interpret the similar structure of the embryo in *Corydalis cava* and *Carum Bulbocastanum* in the same way. With the exception of the slightly bilobed cotyledon found in the embryo of *Ranunculus Ficaria*, the development of all three is precisely alike.

The position of the first leaf, which is opposite the cotyledon, is also an argument for Sterckx's theory. For, if the single cotyledon represents a pair fused along one margin, the first leaf would stand in its proper place relatively to that pair. But if the cotyledon represents one of a pair of which the other has disappeared the abortive cotyledon would stand behind the first leaf, which could not be its original position.

The truth is, however, that there is nothing in the development of the embryo in any one among the three Pseudomonocotyledons just described which will decide between the rival theories. So far as the evidence goes, the single cotyledon in all three might be derived from the ancestral pair either by suppression of one cotyledon or by union of the two into a single member.

But if embryological evidence fails to answer a comparatively simple question such as the method by which two cotyledons in the ancestral *Corydalis*, *Carum*, or *Ranunculus* became reduced to one in certain living species, how can we expect to settle the number of cotyledons possessed by a form so remote as the Primitive Angiosperm by evidence of the same kind drawn from the structure of its living descendants?

So far the comparison with Pseudomonocotyledons has served merely to confirm previous doubts as to the phylogenetic value of characters belonging to the young embryo. It is pretty clear that such characters are mainly adaptive. Ancestral features seem to be obliterated quickly and very completely at that early period. Two causes probably co-operate to this end: the severity of the competition which follows germination, and the plastic nature of the tissues in the young embryo. A very slight start in the race may mean survival to the seedling, and some minute variation in the structure of the mature embryo may ensure such a start. At the same time slight changes in the form of a mass of meristem are easily effected.

What conclusions can be drawn from the history of the embryo within the embryo-sac? We have discussed three possibilities with regard to the number of cotyledons possessed by the Primitive Angiosperms. First, that they had one cotyledon, which gave rise to the pair found in living Dicotyledons by fission. This possibility is usually thought to involve the existence of a truly terminal cotyledon both in the Primitive Angiosperms and in living Monocotyledons. If this were so, we might well hesitate to accept an explanation which involved such a consequence. The difficulties arising out of this hypothesis have already been discussed. But the development of the embryo in the Pseudomonocotyledons examined by Hegelmaier, Schmid, and Sterckx, and in genera from the Dioscoreaceae and Commelinaceae described by Solms-Laubach, shows how a cotyledon of undoubtedly lateral origin may assume a terminal appearance, pushing the stem-apex to one side. The apparently terminal cotyledon of *Alisma* and other Monocotyledons may be derived from a single lateral cotyledon through intermediate forms of this kind.

The possibility of a monocotylar race of Primitive Angiosperms need not then be abandoned on the ground that their single cotyledon must needs be terminal. Nor is it impossible to derive a dicotylar embryo from such an ancestor. The single cotyledon might divide to form a pair of members which by lateral shifting would come to occupy the same relative position as the cotyledons of living Dicotyledons. The view so amended has, however, lost its chief recommendation—that of simplicity. The cotyledons of *Capsella* can no longer be derived by fission only from the single member found in *Alisma*.

Suppose, on the other hand, that the Primitive Angiosperms possessed two cotyledons. They may have been reduced to one in living Monocotyledons by either of two methods. One of the pair may have been suppressed, or both cotyledons may have fused into one. That the change from a dicotylar to a monocotylar embryo can be effected without difficulty is clear from the existence of pseudomonocotyledons—species with a single cotyledon belonging to widely separated Dicotyledonous genera. In each genus the reduction of two cotyledons to one has taken place independently. We have seen that the development of the embryo within the embryo-sac gives no clue to the method by which such reduction takes place—at least in the three species investigated.

Vestiges of a second cotyledon opposite the functional one have been sought in vain by many observers; Hegelmaier believed he had found such a rudiment in the abnormal embryo of *Carum Bulbocastanum* already mentioned. Little weight need be attached to an isolated instance of this kind. The epiblast of certain Grass embryos has been interpreted as a rudimentary cotyledon (Van Tieghem), but this view is not generally accepted.

Little evidence has so far been brought forward in favour of the third and last possibility, that of the fusion of both cotyledons of the Primitive Angiosperm into a single member. Sterckx attributes the bilobing of the embryo in *Ranunculus Ficaria* to an origin of this kind, but even if his arguments were conclusive they would not decide the question in the case of true Monocotyledons.

To sum up, comparison with Gymnosperms establishes a presumption in favour of two cotyledons among the Primitive Angiosperms. The development of the embryo within the embryo-sac gives no decided clue to the ancestral form. The shape of the whole structure and the sequence of its development seem far more dependent on the environment than on inheritance of ancestral features.

One class of evidence is as yet untouched—the history of the embryo after germination. The embryo is less cramped when it has escaped from the seed, and the development of vascular tissue gives rigidity to its form. Ancestral features if reproduced after this epoch stand a better chance of being preserved.

Embryology after Germination.

The anatomy of seedlings cannot be understood apart from their external characters. On germination the outline of the embryo is much altered, but this depends not on the formation of new members but on changes in the proportion of those already present to each other. This is quite clear in the familiar examples of germination—bean, acorn, maize. The exit of the radicle and plumule from the seed is commonly due to the basal elongation of the cotyledon or cotyledons. This elongation is followed,

perhaps accompanied or even replaced, by that of the hypocotyl. Then follows the lengthening of the primary root, and finally that of the plumule, or of the first leaves. This sequence is the rule in Monocotyledons and Dicotyledons alike, but, of course, exceptions are found in both classes. Seedlings adapt themselves in this as in other respects to the demands of their environment.

In every seedling there is a period—longer in some species, shorter in others—during which it consists of cotyledons, hypocotyl, and primary root. The plumular bud is of course present, but neither its axis nor the petioles of its leaves have begun to elongate. It is insignificant compared to the cotyledons. During this period the vascular tissue within the seedling becomes well-defined for the first time. Lignification of the xylem sets in —a change of great importance to the microscopist, since it permits him to pick out the first xylem elements with certainty from other constituents of the bundle. In many slow-growing seedlings the vascular skeleton of the cotyledons, hypocotyl, and primary root, is fairly complete before the plumular traces are well differentiated. In such forms this vascular skeleton is often found to be characteristic of the genus, or even of a larger group. In other words, the skeleton is formed on the same type throughout the genus or the order. Simple as this skeleton necessarily is, more variation in detail is possible within it than might be expected. The number of traces furnished by each cotyledon, their behaviour in the hypocotyl during the transition to a root-structure, the symmetry of the primary root, are all variable characters. Very well-marked types of vascular symmetry can be distinguished, and some of these are clearly derived from others. Thus extended observations show some types to be more primitive than others.

The seedlings of Monocotyledons and Dicotyledons have been examined of late years with a phylogenetic aim. General conclusions have been drawn from comparison of the several types of vascular symmetry found within each class. If such investigations should show that there is a primitive type in each class, we may fairly suppose it to represent the seedling skeleton of the ancestral Dicotyledon or the ancestral Monocotyledon. But of course the primitive or ancestral Dicotyledon and the primitive Monocotyledon are nearer to their common ancestor than living Dicotyledons and living Monocotyledons are. Therefore the primitive forms in either class should resemble each other more closely than living Dicotyledons resemble living Monocotyledons.

Some fairly extensive observations on the vascular symmetry of seedling Monocotyledons have shown that one particular type of monocotylar symmetry may be considered relatively primitive (Sargant, 72). A certain dicotylar type has also been picked out as relatively primitive by other observers, partly by comparison of Dicotyledonous seedlings with each other, but chiefly as the result of comparison with Gymnosperms

(Tansley and Thomas, 87 ; see also Thomas, 88). If these observations are well-founded, the two primitive types ought to resemble each other. If they do, the results in either case are strongly confirmed. If they do not, there must be some flaw in the reasoning on one side or on both.

This test has in fact proved satisfactory. The two types of vascular structure resemble each other very closely. The points which they have in common represent, no doubt, the seedling structure of the Primitive Angiosperms. But the important feature for our present purpose is this. The seedling structure of the primitive Monocotyledon resembles that of the primitive Dicotyledon in its dual symmetry. In other words, judging by its vascular skeleton, the seedling of the Primitive Angiosperm was dicotylar.

The importance of this result renders an examination of the evidence on which it is based imperative. The account which follows is necessarily a mere sketch. For convenience it begins with the Dicotyledons. The external features of their seedlings are more familiar to botanists, a greater mass of evidence concerning their internal structure is available, and their vascular symmetry is less variable and more easily described.

The most important series of researches hitherto made on the anatomy of Dicotyledonous seedlings from a phylogenetic standpoint is that carried out by Mr. A. G. Tansley and Miss Thomas. It is as yet published only in abstract. But in the light of their results previous work such as that of Gérard (27), Dangeard (20), and others acquires a new value. The seedling anatomy of Dicotyledons had been examined much more fully than that of Monocotyledons before the microtome came into common use. The suppression of the internodes, which is characteristic of Monocotyledonous seedlings, was indeed a formidable obstacle to research when the only possible method of examination was by successive hand-sections. But hand-sections could be used for the fairly long internodes of Dicotyledons.

Thus Gérard, in his elaborate memoir of 1881 (27), described fifty-seven species of Dicotyledons, giving every detail of the transition from root to stem in the young seedling, but he attacked only nine species of Monocotyledons. And as these were necessarily chosen because they possessed fairly long hypocotyls and plumular internodes, they did not properly represent their class.

Dangeard gave a more general sketch of the anatomy of Dicotyledonous seedlings in 1889 (20), and we have, in addition, accounts of isolated families or genera by Van Tieghem, Quéva, Sterckx, Chauveaud and others. These researches have for the most part been directed to an anatomical end—the description of the transition from root to stem-structure in the hypocotyl.

The evidence then to which I shall refer is, in the first place, that collected by Mr. Tansley and Miss Thomas. In the account which follows I have drawn largely on both the published abstracts dealing with their work

(87, 88). The generosity of the authors has allowed me to verify details by examination of their original preparations. Their researches deal, as already stated, with Gymnosperms as well as Dicotyledons.

Next in importance comes recent work inspired by the same phylogenetic aim, but dealing with comparatively small groups (T. G. Hill, 40, 41; A. W. Hill, 39; Matte, 60; Hill and de Fraine, 42). Finally we have the anatomical literature just quoted as corroborative evidence.

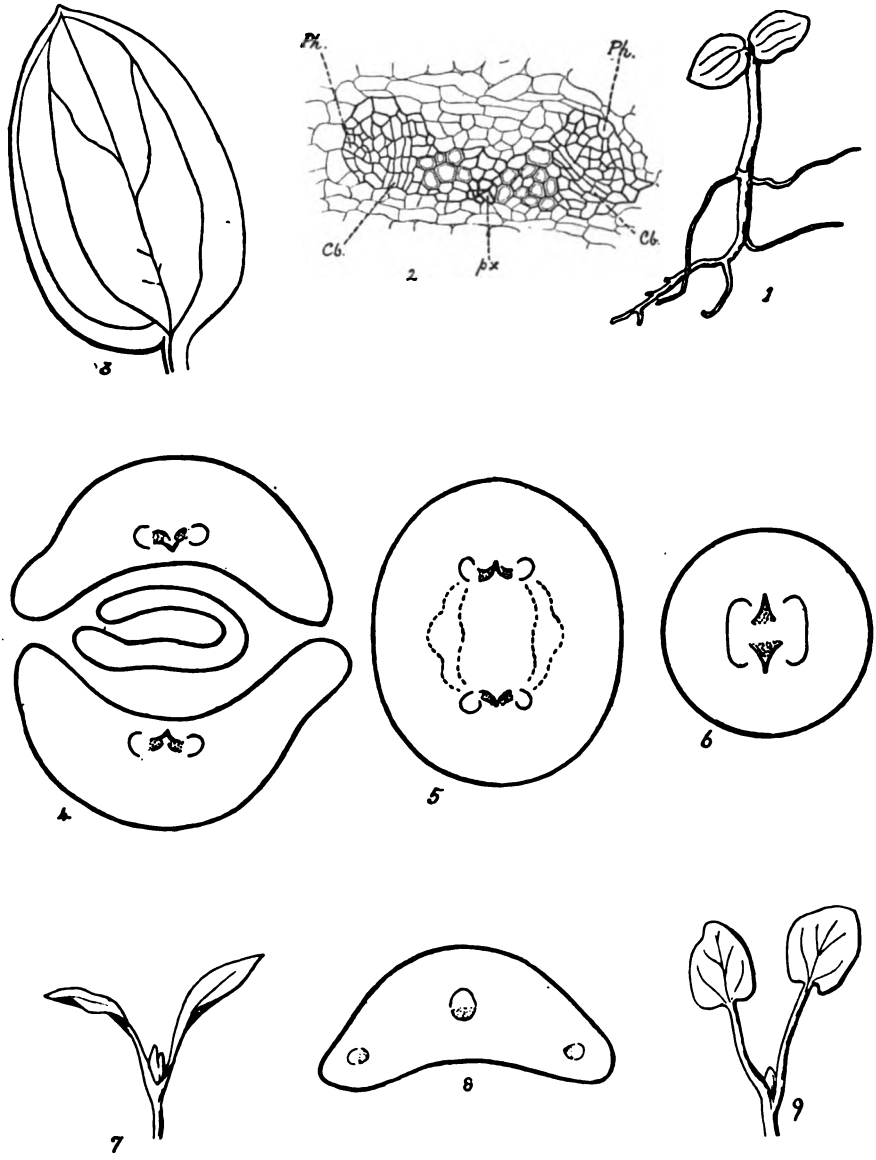
After my own experience of Monocotyledons with their endless variety in detail, the uniformity of Dicotyledons is very striking. The hypocotyl and primary root form part of the main axis of the plant. In annuals, biennials, some perennials, and in most trees, they last its life. They commonly survive for several seasons even when not permanent. Accordingly these organs become adapted to their functions in a way rarely found among Monocotyledons. The survival of variations in their structure depends on conditions affecting the plant during its whole life, not on those alone which immediately succeed its germination. We have in consequence a tendency to uniformity in the primary structure of the hypocotyl and primary root, because it reflects the permanent conditions of life, and these are uniform, too, in the long run. Moreover, in Dicotyledons the primary structure of root and stem alike is subordinate to the secondary structure, and this is uniform throughout the class.

Accordingly the Dicotyledons as yet examined display but two distinct types of seedling structure. Both are widely distributed within the group. The extreme forms of each type are connected by intermediate links. The types may be distinguished as tetrarch and diarch (Thomas, 88, pp. 79–81; Dangeard, 20).

In both types the blade of each cotyledon possesses a midrib, and, as a rule, two main lateral bundles. All three bundles enter the petiole and commonly fuse there, but occasionally they are found as distinct traces within the hypocotyl, and do not unite even during their insertion on the stele.

In the diarch type the lateral traces always unite with the midrib before its insertion on the stele, though complete fusion is sometimes postponed very late. Mechanical reasons may sometimes determine the level of the junction. Thus in *Nigella damascena* the petioles of the cotyledons are quite 10 mm. long (Fig. 7). The midrib runs down the middle of each petiole, and a lateral bundle stiffens either edge (Fig. 8). In the much shorter petioles of *Delphinium requienii* (Fig. 1) the laterals are inserted on the midrib at the base of the blade (Fig. 3). The fusion bundle can be followed down the petiole, and in transverse section it shows the curious double structure (Fig. 2). The phloem groups are distinct: the metaxylem groups are partially divided from each other by a single group of protoxylem, common to both divisions of the bundle. In *Nigella* the midrib trace shows a similar double structure at the base of the petiole

just before the insertion of the lateral traces on it. When this insertion takes place, which is not until the three traces are close to the stele of the hypocotyl, the symmetry of the midrib-trace is not affected.



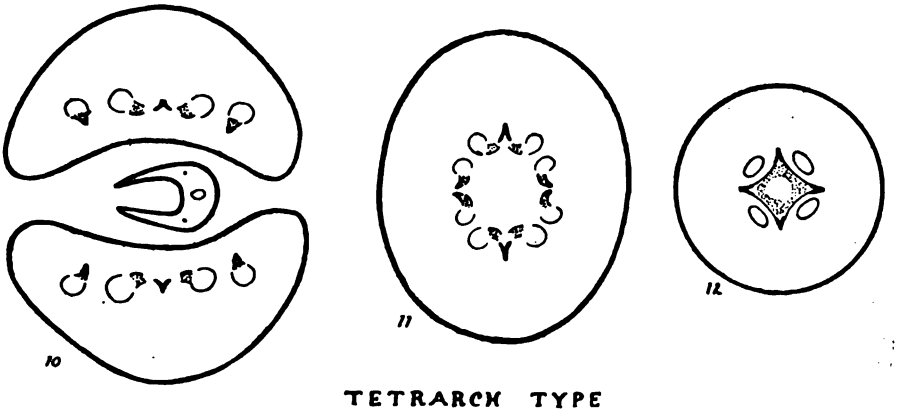
FIGS. 1–6. *Delphinium requienii*: 1, seedling, showing nearly sessile cotyledons; 2, double bundle from petiole of cotyledon; 3, cotyledon (enlarged), showing venation; 4–6, diagrams of transition from stem to root. Here, as in later diagrams, protoxylem is black, metaxylem dotted, and phloem merely outlined.

FIGS. 7, 8. *Nigella damascena*: 7, upper part of seedling; 8, transverse section of petiole of cotyledon.

FIG. 9. *Althea rosea*: upper part of seedling.

The protoxylem of the double trace is often completely external by the time it enters the stele of the hypocotyl, and the transition to a diarch root-stele is then very simple (Figs. 4–6), though commonly delayed for a short distance by the insertion of plumular traces (Fig. 5). These traces do not affect the symmetry of the stele in the hypocotyl and root. In *Delphinium* and *Nigella*, as in most seedlings with elongated hypocotyls among the Ranunculaceae, the stele becomes quite root-like high up in the hypocotyl. No further change is necessary as it passes into the primary root. In other families, however, the transition is often prolonged.

In *Nigella* it is clear that the double structure belongs to the midrib only. The lateral traces when inserted on it do not affect its symmetry. By analogy we must suppose that the double structure of the trace in *Delphinium* also is due to the influence of the midrib.



FIGS. 10–12. Tetrarch type of vascular symmetry, adapted from Miss Thomas's diagram (88, p. 81): 10, cotyledons with first leaf between them; 11, hypocotyl; 12, primary root.

In the extreme form of the tetrarch type, as described by Miss Thomas (88, p. 81), the midrib is represented in the petiole by two distinct bundles. As two laterals are also present, the petiole of each cotyledon contains four bundles, and these enter the hypocotyl as distinct traces. The stele of the hypocotyl receives eight cotyledonary traces. The two pairs which represent the midrib-traces in such a form as *Nigella* behave exactly as in the diarch type. The protoxylem groups of each pair form a single protoxylem ray of the tetrarch root-stele. The right-hand lateral of one cotyledon joins with the adjacent lateral of the other to form a third protoxylem ray, and the left-hand laterals form a fourth in the same way (Figs. 10–12).

This extreme case is less frequent than the symmetry described by Gérard in *Althea rosea*, a form also included within the tetrarch type of Mr. Tansley and Miss Thomas (87, also 88, p. 80). The bundles from the blade approach each other very closely in the long petiole of the cotyledon

(Fig. 9). The laterals are in contact with the double trace of the midrib, though they have not fused with it (Fig. 13, p. 170). The trace which enters the stele of the hypocotyl consists of a median double bundle with external protoxylem. A lateral bundle is in close contact with it on either side (cf. Gérard, 27, Fig. 24). The transition to a tetrarch root-stele is in essentials the same as that of the previous type (Figs. 14, 15; and Gérard, 27, Figs. 23–21). This type differs from the extreme forms previously described only in the closer approximation of the bundles in the petiole of each cotyledon.

In the upper part of its vascular skeleton, the seedling of *Althea* does not differ greatly from that of *Nigella*. Each of the twin compound traces entering the hypocotyl from the cotyledons consists in both genera of a median double trace with a lateral trace approaching it on either side. In *Nigella* fusion of each pair of laterals with its median trace is complete though tardy. The root-stele is diarch. In *Althea* the laterals preserve their independence throughout the transition, and furnish an additional pair of protoxylem rays to the root, which is consequently tetrarch. *Althea* and the seedlings which resemble it clearly belong to the tetrarch type, but they show some approach to the diarch symmetry. *Liriodendron*, as figured by Miss Thomas (88, p. 81), though diarch, makes an even more decided approach to tetrarch symmetry.

Here then is a series of forms linking the extreme tetrarch type (Figs. 10–12) to the extreme diarch (Figs. 4–6). Which of the two is the more primitive? Is the tetrarch type derived from the diarch, or the diarch from the tetrarch?

The authors to whom I have been referring answer this question in their joint note (87). Miss Thomas gives their reasons more fully (88). They do not consider that comparison of different Dicotyledons with each other gives any decided clue. Both types are found in a wide range of families; some in either list are probably ancient. But comparison with the seedlings of Gymnosperms is more instructive. Again both types are found, but here the tetrarch symmetry is characteristic of those Gymnosperms which botanists agree to consider primitive on other grounds.

The seedling of the primitive Gymnosperm, then, probably possessed tetrarch vascular symmetry. But it must not be forgotten that a gap of enormous width separates the primitive Gymnosperm from modern Angiosperms. Reference to the skeleton pedigree reproduced on page 137 shows the latest common ancestor of both groups to be some unknown Pteridosperm. Assuming that Cycads and other primitive Gymnosperms derived the tetrarch symmetry of their seedlings from this Pteridospermic ancestor, we make a second and a greater assumption in supposing that the tetrarch symmetry of modern Dicotyledons has come down unchanged through countless generations of Hemiangiosperms to the Primitive Angiosperms,

and thence through a shorter but still very long period to some living Dicotyledons.

Considering the enormously long epochs with which we are dealing, it might be argued that the two cotyledons of modern Dicotyledons, and the tetrarch skeleton found in many of their seedlings, are not derived from the same source as those characters in living Cycads. The ancestral Pteridosperm may not have possessed them, or if it did perhaps they were lost during the gradual evolution of Primitive Angiosperms through a series of Hemiangiospermous forms. In either case these characters would not have been handed down directly from Pteridosperms to modern Dicotyledons on the one hand and Cycads on the other.

Even on the evidence already given, it must be admitted, however, that the theory of a common origin for these characters supplies the simplest explanation of the facts. The burden of proof lies with those who dispute it. But this is not all. There is evidence from other sources to show that the seedlings of Primitive Angiosperms possessed two cotyledons and tetrarch symmetry.

The Bennettiteae probably diverged from the main Angiospermous line of descent at a later period than the Cycads. But the embryo of the Bennettitean seed is always dicotylar. This increases the presumption derived from the two cotyledons of Cycads, that the descendants of the ancestral Pteridosperm were dicotylar too, and suggests that they retained that character at least as late as the period at which the Bennettitean race was given off. This brings us nearer to the Primitive Angiosperms on one side. On the other lie the Primitive Dicotyledons and the Primitive Monocotyledons. So far as internal evidence goes, the seedling of the Primitive Dicotyledon may have shown either diarch or tetrarch symmetry. What is known about the seedling of the Primitive Monocotyledon?

Among Monocotyledons as among Dicotyledons there is a period in the history of the seedling during which it consists of cotyledon, hypocotyl, and primary root. The plumular bud is still quite embryonic, and is as a rule completely enclosed by the base of the cotyledon, or by a sheath-like appendage attached to it. But in Monocotyledons the primary root as well as the single cotyledon is commonly a short-lived structure. The hypocotyl is almost always much reduced in length, even when it is not transformed into a tuber. The young seedling then to all appearance consists only of cotyledon and primary root—two short-lived members separated by a brief region representing the hypocotylar axis. In many genera this region hardly exists at all; the cotyledon seems to pass directly into the primary root. The early internodes of the stem are frequently much suppressed too, and the early leaves then appear directly connected with the first cauline roots. Such leaves do not receive their water-supply through the main axis

as in Dicotyledons, but directly from the roots to which they are respectively attached.

The primary root then naturally withers away at the same time with the cotyledon. They rarely survive the first season of growth, and their structure—external and internal—is therefore affected by those conditions only which immediately succeed germination. With the later life of the plant they have nothing to do. This peculiarity accounts for the much greater variety of internal structure found in the young seedling of Monocotyledons as compared with that of Dicotyledons.

In consequence of this variety the results of such an examination cannot be so clearly defined. But, on the other hand, the vascular characters of the seedling are of more taxonomic value among Monocotyledons than among Dicotyledons. A genus or a group of genera is very often separated from its fellows by the characteristic vascular skeleton of its seedling.

Two distinct types of vascular skeleton are often connected with each other by a series of intermediate forms. When such forms constitute a single series—such, for instance, as that connecting the diarch with the tetrarch type among Dicotyledons—there is usually no way of determining which of the two extreme cases is the more primitive. But when, as sometimes happens, several series start from a single type, when, that is, this single type is connected with two or more very distinct forms by two or more distinct series of links; then there is very good ground for supposing the type common to all the series to be primitive. For it is generally acknowledged that the descendants of a primitive stock may be modified in various ways, until several races are evolved which differ very considerably from each other both externally and internally. But it is far more difficult to imagine causes sufficiently potent to lead even two distinct forms to complete identity in internal structure. And this difficulty is enormously increased when we start with more than two.

By comparison of seedling symmetry in the genera belonging to a single family, it is thus possible to pick out a type of seedling structure as relatively primitive within that family. This has been attempted in the Liliaceae (Sargant, 72). The seedling of *Anemarrhena*, a monotypic genus belonging to the Asphodeleae, possesses a very definite and characteristic vascular skeleton (Sargant, 70). The vascular skeletons of seedlings belonging to other genera of the Asphodeleae can be derived from the *Anemarrhena* type; many of them are, indeed, merely variants on it. The vascular symmetry of seedlings belonging to another tribe, the Allieae, is linked to the *Anemarrhena* type through *Arthropodium*. Thus comparative evidence suggests that *Anemarrhena* is primitive as compared with other genera found in these two tribes. But this is not all. The *Anemarrhena* structure reappears in *Galtonia* and *Albuca* which belong to the Scilleae. The very numerous types of vascular symmetry found within this tribe, and also the

type characteristic of the Tulipeae, can be derived from the seedling skeleton of *Albuca* or *Galtonia*, which must therefore be primitive in these tribes too.

Thus the various types of seedling symmetry which have been found within the four tribes, Asphodeleae, Alliceae, Scilleae, and Tulipeae, appear to be founded on the *Anemarrhena* skeleton. But these four tribes occupy a central position within the family. Together with the Hemerocalleae, the Aloineae, the Yuccoideae, and the little-known Johnsonieae (or Aphyllantheae), they constitute the Liliaceae in the narrowest sense. The eight tribes just mentioned are united by Mr. J. G. Baker in the sub-order Liliaceae verae (6, pp. 354–6, and 7, pp. 209, 210). The seedlings of the Aloineae and Yuccoideae which I have examined have been so far modified by the conditions under which they germinate that most primitive features are lost (72, p. 34). *Hemerocallis fulva* shows a general resemblance to the stouter Scilleae, such as *Eucomis*. Nothing is known of seedling anatomy in the Johnsonieae. So far, then, it would seem that the *Anemarrhena* seedling presents the most ancient type of vascular symmetry found among the Liliaceae proper.

The other tribes included among the Liliaceae by Baillon (5) or Bentham and Hooker have all been treated at some time or other as belonging to distinct families. Thus in the English translation of Le Maout and Decaisne's textbook (52, p. 846) the family Liliaceae corresponds pretty closely to Mr. Baker's sub-order Liliaceae verae, and the Junceae, Aspidistreae, Melanthaceae, Smilaceae and Asparageae are treated as distinct families. A note is added to the Liliaceae: 'We have indicated the extremely close affinities between Liliaceae, Asparageae, and Smilaceae; families which together form a group to which most other Monocotyledonous families may be linked, directly or by intermediates. Thus Junceae, which are near certain Melanthaceae and Liliaceae, connect these with other families with a free ovary; and, on the other hand, those Amaryllideae and Dioscoreae which belong, the one to the Liliaceae, the other to the Smilaceae with an inferior ovary, connect them with the epigynous Monocotyledons.'

The seedling structure of Monocotyledons, so far as it has been examined, is not inconsistent with the view expressed in this quotation. The outlying tribes of Liliaceae in the larger sense possess seedlings whose vascular symmetry can be referred to one type or another belonging to one of the four central tribes. The Amaryllidaceae, Iridaceae, and Aroideae, seem similarly attached by seedling characters to the Liliaceae proper. The seedlings of Palmae, Scitamineae, and Gramineae, indeed, are very distinct, but they are all much modified forms, and there is nothing in their seedling structure to suggest a primitive symmetry other than that of *Anemarrhena*. Indeed, many points in the vascular skeleton of *Elettaria*

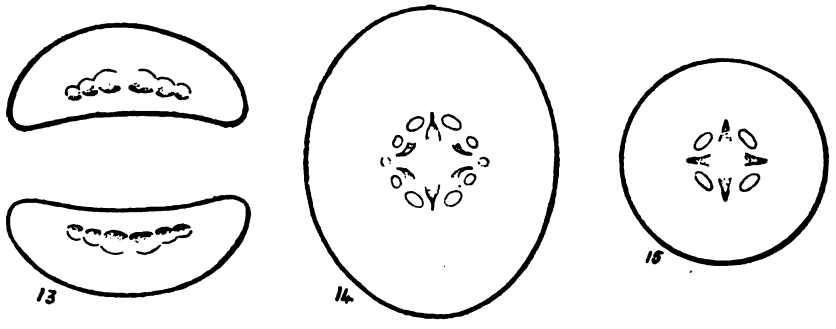
and other genera of the Scitamineae recall the tetrarch symmetry of *Anemarrhena* (72, pp. 38–40, and pp. 50–52).

The *Anemarrhena* type, then, may very well be primitive among Monocotyledons in general as well as among the Liliaceae proper. But if so, it will probably reproduce the main features of the seedling in the primitive Monocotyledons. The exact structure of this type is therefore of importance in the present inquiry.

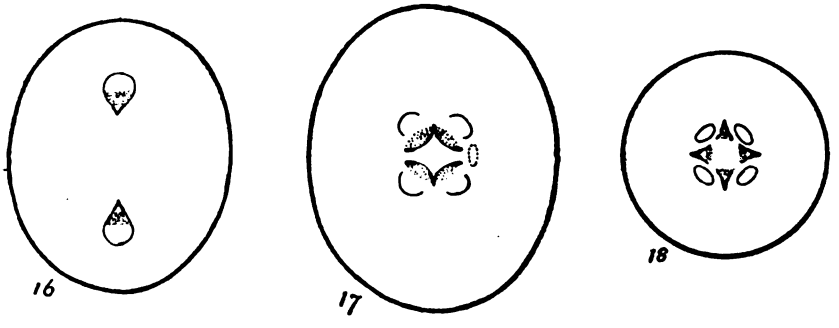
The cotyledon in *Anemarrhena*, *Albuca*, and *Galtonia* is erect and green. The apex is enclosed within the seed, and commonly carries the shell of the seed above ground with it (Sargant, 70 and 72). The primary root is long and stout; externally it appears as the direct prolongation of the cotyledon, from which it is divided only by the slight swelling which marks the position of the future bulb or tuber. Two massive bundles traverse the cotyledon from apex to base. Above the plumular bud they occupy the foci of the elliptical transverse section (Fig. 16). They are symmetrically placed in the sheath and below the plumular bud—the traces of which remain embryonic long after germination—the two cotyledonary traces meet in the centre of the circular section to form the stele of the hypocotyl (Fig. 17). As they approach, each trace opens out into a double bundle, resembling in essentials that of *Delphinium* (Fig. 2). Before the double traces meet, the xylem of each branches in three directions (Fig. 17), and for a very short distance there are six protoxylem rays and four phloem groups in the stele. Below this the stele becomes root-like and tetrarch by union of two pairs of adjacent lateral protoxylems (Fig. 18).

The resemblance to the vascular skeleton of *Althea* needs no comment. It is startling when the diagrams are compared (Figs. 13–15 and 16–18). But *Althea* is a mere variant, very slightly reduced, on the tetrarch structure which is certainly primitive among Gymnosperms (Figs. 10–12), and probably primitive among Dicotyledons. We have seen that the seedlings of primitive Dicotyledons must have been either diarch or tetrarch in their vascular symmetry. Comparison with the seedlings of Gymnosperms inclines the scale in favour of the tetrarch type. This comparison, however, cannot be held to settle the question, because living Cycads are very remote from living Dicotyledons (*ante*, p. 165). The presumption is undoubtedly in favour of the tetrarch symmetry of seedlings among the primitive Dicotyledons.

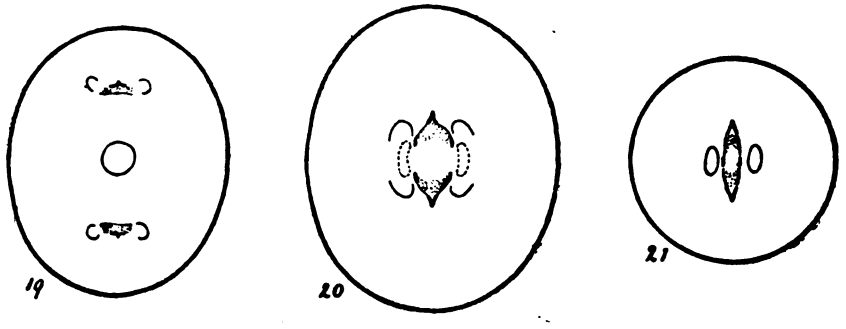
This presumption is increased almost to certainty by comparison with Monocotyledons. We have seen that the tetrarch type of symmetry reappears among them. It reappears in seedlings believed on independent grounds to represent a type of vascular symmetry primitive among Monocotyledons. In other words, the evidence suggests that the seedling of the primitive Monocotyledon was tetrarch.



ALTHEA ROSEA



ANEMARRHENA ASPHODELOIDES



PODOPHYLLUM PELTATUM

FIGS. 13–21. Three series of diagrams showing transition from stem to root structure. In each series the first section passes through the petiole of each cotyledon above the stem-bud, the second through the hypocotyl, and the third through the primary root, just below the transitional region.

FIGS. 13–15, *Althea rosea*; 16–18, *Anemarrhena asphodeloides*; 19–21, *Podophyllum peltatum*.

This similarity in structure between the seedling of the primitive Monocotyledon and that of the primitive Dicotyledon is precisely what was foreseen on theoretical grounds (*ante*, p. 160). It confirms the results derived from study of the evidence relating to both classes. It is completely and simply explained by supposing the seedling of the Primitive Angiosperm to have possessed tetrarch vascular symmetry which it handed down with more or less modification to its immediate descendants, monocotylar or dicotylar.

If this be accepted as a working hypothesis, does it follow that the embryo and seedling of the Primitive Angiosperms had two cotyledons? The seedling of *Anemarrhena* has but one, yet its tetrarch symmetry is very clear. Might not a monocotylar Primitive Angiosperm give rise to tetrarch dicotylar forms, as well as a dicotylar Primitive Angiosperm to tetrarch monocotylar forms?

If the alternatives are followed out to their consequences, the second hypothesis—that of the dicotylar Primitive Angiosperm—is certainly far more probable. Any stele which receives traces from two similar and opposite cotyledons, each symmetrical about the same median plane, must itself be symmetrical about two vertical planes: that is, about the median plane common to both cotyledons, and about the intercotyledonary plane perpendicular to it. The reduced or diarch type of skeleton described in the seedlings of *Delphinium* and *Nigella* is as completely symmetrical about both planes as the tetrarch skeleton of *Althea*. Reduction within the seedlings of Dicotyledons does not tend to produce a skeleton symmetrical about one plane only. On the other hand, a stele receiving traces from a single lateral member is naturally symmetrical about the median plane of that member, but not necessarily or even probably symmetrical about the vertical plane at right angles to it.

Accordingly, among Monocotyledons, the tetrarch skeleton as it is found in *Anemarrhena*, *Albuca*, and *Galtonia*, is the only type symmetrical about two planes. Even in these cases symmetry about the plane bisecting the cotyledonary traces is not quite complete, for these traces are slightly displaced in one region of the axis by the insertion of the plumule (Sargant, 72, Diagram VI (A), pp. 26 and 70, Fig. 3).

That the tetrarch skeleton, symmetrical about two vertical planes, should be found in Monocotyledonous seedlings of an ancient type is exactly what would occur if the primitive Monocotyledon were derived from a dicotylar ancestor by the fusion of both cotyledons into a single member. The dual symmetry of the internal structure would naturally survive the external bisymmetry. On this hypothesis we may suppose that two cotyledons were characteristic of the main line of Angiospermic descent from the original Pteridosperm down to the evolution of the primitive

Monocotyledon. The two cotyledons of Cycads and of the Bennettiteae would be derived from ancestors in that line of descent.

But the case is very different if we are to postulate a monocotylar Primitive Angiosperm. We must then either give up the connexion with the Cycads and the Bennettiteae altogether, or suppose that the monocotylar form of seedling was evolved in an ancestor of the Primitive Angiosperms more recent than that which they have in common with the Bennettiteae. From the monocotylar seedling of Primitive Angiosperms derived in this way, a dicotylar race must be again evolved from which modern Dicotyledons are descended. Further, some descendants of this comparatively modern dicotylar race must reproduce the tetrarch seedling structure of the Cycads. The least improbable form which this hypothesis could take would be to suppose the seedling of Primitive Angiosperms to resemble that of *Anemarrhena*. That is, it would be completely monocotylar externally, but its vascular skeleton would remain tetrarch. But if we are to credit the Primitive Angiosperms with this partial assumption of monocotylar symmetry, we burden ourselves with the necessity of explaining why the main body of its descendants should have reverted to the dicotylar form. All these difficulties are escaped if we suppose the Primitive Angiosperms to have possessed two cotyledons, united in living Monocotyledons to form a single member.

The antiquity of the dicotylar type is suggested by all the evidence which we possess at present. But so far the suggestion that the pair of cotyledons characteristic of Primitive Angiosperms united to form a single member in one branch of its descendants depends only on the evidence for the comparative antiquity of the *Anemarrhena* type of vascular symmetry. This is, indeed, the chief argument in its favour. But corroborative evidence is found in the vascular structure of some Ranal seedlings with cotyledons united almost to the top. *Eranthis hiemalis* has been described elsewhere (Sargant, 71 and 72). Its seedling skeleton is very similar to that of *Anemarrhena*. The stele of the primary root is indeed diarch in *Eranthis*. But at the level where the root meets the tuberous hypocotyl there are marked indications of a tetrarch structure. *Podophyllum peltatum* has a similar seedling (Holm, 45). Its anatomy has not hitherto been described in detail. The main features of its vascular skeleton are given in Figs. 19–21 (p. 170).

Just above the plumular bud the united petioles of the cotyledons are almost solid. The outline of the section is elliptical, the bore of the tube very narrow, and the two traces occupy the foci of the ellipse. Each trace is clearly double: the protoxylem is already becoming external (Fig. 19). The hypocotyl is not swollen into a tuber, though its tissues are packed with starch. As the cotyledonary traces approach the centre, they are

joined by traces from the rudimentary plumule. The protoxylem of each cotyledonary trace branches in three directions: the four phloem groups remain distinct for some time. The resemblance of the stele at this level in transverse section (Fig. 20) to that of *Anemarrhena* (Fig. 17), and *Althea* (Fig. 14), is very clear. But in *Podophyllum* as in *Eranthis* the four phloem groups do not remain distinct. They unite in pairs lower down, opposite the intercotyledonary poles of protoxylem (Fig. 21), and the latter finally disappear, leaving a completely diarch root-stele.

In *Podophyllum* and *Eranthis* the two cotyledons are united for at least four-fifths of their whole length, and in both the vascular skeleton shows signs of reduction from the tetrarch type. This reduction seems to have begun as in *Althea*, but to have proceeded further. The fusion of the lateral traces with the midrib is more complete in the petioles. The formation of a tetrarch root-stele is merely indicated: the final form is diarch.

Now the *Anemarrhena* symmetry can also be considered as a reduction from the *Althea* type. The reduction in the root-stele is less complete than that of *Podophyllum*. On the other hand, the bundles of the petiole—so clearly double in *Podophyllum* (Fig. 19)—are single in *Anemarrhena* (Fig. 16): their double structure is indicated only just before the transition begins. In spite of these differences, the general resemblance between the vascular skeleton of the *Anemarrhena* seedling and that of *Podophyllum* or *Eranthis* cannot be denied. It appears, then, that the partial union of cotyledons in a tetrarch seedling has actually operated to reduce the vascular skeleton to a form very near the *Anemarrhena* type. Whether the facts imply any real relationship between the Ranales and the Liliaceae or not, they do at least show that union of the cotyledons in a tetrarch dicotylar seedling might bring about the formation of a monocotylar type with a reduced vascular skeleton resembling that of *Anemarrhena*.

Phylogenetic Schemes.

The two characters which separate Monocotyledons from Dicotyledons most completely have now been discussed. Primitive Angiosperms appear to have resembled their Dicotyledonous descendants in both. There can be little doubt that they possessed a cambium. The evidence is perhaps less conclusive with regard to the number of cotyledons, but still there seems good reason to think that Primitive Angiosperms had two. The embryological evidence on which this conclusion is chiefly based requires also that the single cotyledon of Monocotyledons should be regarded as a fusion of the ancestral pair.

Monocotyledons, however, differ from Dicotyledons in a number of other characters. From the systematic point of view they are of minor importance, because they are far less constant than those already discussed. Leaves with parallel venation are, for example, the rule among Mono-

cotyledons and rather rare among Dicotyledons, but quite a large number of Monocotyledons have net-veined leaves. Again, the three-fold symmetry of the flower is quite characteristic of Monocotyledons, but some Dicotyledons display it, while many Monocotyledons have whorls of four or even five parts. So with other characters, such as the short-lived primary root of Monocotyledons and their albuminous seeds. Though no one of these minor characters can be used as a test in the same way as the stem-anatomy or the number of cotyledons, yet collectively they do separate Monocotyledons from Dicotyledons very completely.

This consideration suggests another way of attacking the whole problem. Whichever view we take, for example, concerning the number of cotyledons possessed by the Primitive Angiosperms, whether with Professor Lyon we suppose it monocotylar, or dicotylar with Professor Henslow, one branch of its descendants must be considered as inheriting the original character, and the other as modified in that respect. If the seedling of Primitive Angiosperms was monocotylar, then the two cotyledons of Dicotyledons were developed from the single ancestral member. If the Primitive Angiosperms had two cotyledons, then the single member of Monocotyledons is the innovation.

In either case some cause must have existed for the change. The variations in that direction would not have survived had not circumstances prevailed under which the new structure was in some respects preferable to the old. And it is probable that some of the other characters now associated with the possession of one cotyledon, or of two, were differentiated in response to the same circumstances.

We may, then, inquire whether any causes have been suggested to account for the formation of two cotyledons from one or one from two. And if more than one view has been advanced, which of them would best explain the correlation of stem-anatomy and minor characters with the derived form?

To begin with the theory of a primitive single cotyledon giving rise to a pair by fission, how does it stand this test? Professor H. L. Lyon is the most recent exponent of this view. His papers (57, 58) are remarkable for the precision of their statements, and for the unflinching way in which he faces the theoretical consequences of his views. But the only hint given as to the cause of the fission by which one cotyledon becomes two is the suggestion that a bifid cotyledon might be of use to the embryo while it is getting clear of the seed. No attempt is made to correlate the stem anatomy of Dicotyledons, or any of their minor characters, with the dicotylar form of their seedlings.

This omission is not peculiar to Professor Lyon. The monocotylar theory has been accepted by most botanists for more than a generation,

and it has proved barren. No consistent explanation of the derivation of Dicotyledons from a monocotylar race has yet been offered which would account for the difference in stem anatomy and in some minor features which exists between the two classes. This is in itself a strong argument against the truth of the monocotylar view, and serves to confirm the evidence in favour of crediting the Primitive Angiosperms with two cotyledons.

Assuming that the seedling of Primitive Angiosperms was dicotylar, we have to account for the origin of Monocotyledons. Most of the few botanists who upheld this view in the last century supposed the single cotyledon found in this class to represent one of the ancestral pair, the second having been suppressed. Agardh, however, suggested in 1829 that in some Monocotyledonous families the single cotyledon might represent a fusion of the Dicotyledonous pair. This suggestion seems to have been founded partly on a misinterpretation of the seed-structure of *Nymphaea* and its allies, and it fell into oblivion.

The most important paper published of late years in support of the first of these alternatives is that of Professor Henslow (published in the Linnean Journal for 1892 (38)). He assumes a common origin for both classes, and collects a quantity of evidence to show:—

1. That Monocotyledons are derived from a stock essentially Dicotyledonous; that is, possessing two cotyledons, a true cambium, and some of the minor characters of Dicotyledons.

2. That the single cotyledon of Monocotyledons represents one of those possessed by the ancestral stock, the other having been suppressed.

3. That the features characteristic of Monocotyledons were evolved during the adaptation of one or more branches of the ancestral stock to an aquatic habit.

With regard to the first proposition we have seen that existing evidence is altogether in favour of supposing Primitive Angiosperms to have possessed a true cambium and two cotyledons. The evidence advanced in support of the two succeeding propositions may be criticized.

Professor Henslow points out that a very large proportion of Monocotyledonous orders are aquatic—about 33 per cent. as compared with 4 per cent. of Dicotyledonous orders. When a class or an order contains aquatic forms only, it is reasonable to conclude, in the absence of evidence to the contrary, that the common ancestor of that class or order was aquatic also. But the existence of a large minority of aquatic orders within a class may be equally well explained on grounds other than the descent of all members of that class from an aquatic stock. In this case I believe that Monocotyledons may be shown to be on the whole a decadent race of which some branches have been driven to an aquatic habit to escape the severer competition on land.

Monocotyledons are, on the whole, a far less vigorous class than Dicotyledons; as a rule their representatives are found under rather exceptional conditions. Thus Palms do not extend far beyond the tropics; bulbous and tuberous families (Liliaceae, Iridaceae, &c.) are characteristic of Alpine situations and dry climates with periodical rains. Almost the only Monocotyledons which compete successfully with Dicotyledons over a wide range are the Grasses.

Professor Henslow has remarked on the large proportion of small orders found among Monocotyledons. Considering this character, together with the peculiarities of distribution just mentioned, they may perhaps be considered as a class which has seen better days:—as survivals from a period in which they were more numerous and more widely spread than in the present geological epoch. I shall endeavour later on to show that such an hypothesis is not unreasonable; if it be granted, we may look on living Monocotyledons as a race which has been on the whole unsuccessful in the struggle for existence, and in consequence maintains itself chiefly in situations where the local conditions are exceptionally favourable to its peculiar characters.

The number of small aquatic orders found among Monocotyledons is easily explained by this hypothesis, for among animals and plants alike many ancient forms have survived by adapting themselves to life in small ponds or in streams where the competition for existence is less keen than under more genial conditions.

A mass of observations has been collected by Professor Henslow to show that features characteristic of Monocotyledons occur chiefly or exclusively among aquatic Dicotyledons, when found in that class at all. Only a few of these observations can be mentioned here; I have attempted to pick out the most important.

(i) *Arrest of one Cotyledon.*

The examples given are *Trapa natans*, *Ranunculus Ficaria*, and *Carum Bulbocastanum*. Of these only *Trapa natans* is aquatic, and it is, so far as I know, the only instance among aquatic Dicotyledons in which one cotyledon is absent or much reduced. As a rule they possess two, often specialized as food-storers. On the other hand, there are at least twelve other species of Dicotyledons distinguished by possessing one cotyledon only (Sargant, 72, p. 76). Not one of them is aquatic, but they are all geophilous, and most of them very highly specialized to that habit.

(ii) *Sheathing petioles.*

The leaves of Monocotyledons are commonly expanded at the base into a broad sheath, which encloses the bases of the younger leaves more or less completely. The petiole of some Dicotyledons is expanded at the base

in a similar way, and some of these species are aquatic. But sheathing petioles are also found in terrestrial Dicotyledons: for instance, in many Umbellifers.

In Monocotyledons the connexion is clear between the formation of a sheathing petiole and the suppression of stem internodes. Both characters are correlated with the formation of a large bud on a squat subterranean axis—a character so common in this class. The bud is commonly dormant throughout the winter, and sends up leaves and flowers in the spring. In such an axis the internodes are disk-shaped, and for the adequate insertion of radical leaves a broad base is necessary. In a large underground bud, moreover, the protection of one leaf by the expanded base of that outside it is very convenient.

The aquatic Dicotyledons with sheathing petioles are forms such as *Nymphaea*, having rhizomes which creep in the mud at the bottom of the pond and send green shoots upwards into the water. The green shoots die down in the winter like the aerial shoots of geophytes. Moreover the terrestrial Dicotyledons with sheathing petioles are mainly geophytes; that is, species with underground stems which throw up aerial shoots each spring to die down in the winter.

In short, the character which all these Dicotyledons with sheathing petioles have in common is not the aquatic but the geophilous habit. Aquatics, such as *Nymphaea*, which have a permanent axis beneath the mud at the bottom of the ponds where they grow, have some characters in common with geophytes.

(iii) *Stem anatomy.*

Similar criticism applies to Professor Henslow's remarks on the anatomy of the stem. He explains with great clearness how a number of broad-based leaves inserted on a squat axis introduce more traces into that axis than can be accommodated in a single circle. Hence the formation of several concentric circles of traces. Secondary thickening is not usually required in such a stem. The intrafascicular cambium soon disappears, particularly when starch and other food-stuffs are packed in the conjunctive tissue which separates the bundles. But traces of cambium within each bundle are commonly found in geophilous Dicotyledons.

But though this explanation may—and probably does—account for the scattered arrangement of bundles in the rhizomes of many aquatic Dicotyledons, the character belongs exclusively to the permanent axis rooted in the soil beneath the water. The vascular tissue of the axes which grow upwards into the water is always very much reduced in quantity. The stele of completely submerged stems commonly becomes a slender cylinder in which the leaf-traces lose their identity.

Thus the likeness between the stem anatomy of aquatic Dicotyledons and that characteristic of Monocotyledons in general is confined to the

underground axes of the former. That likeness is shared by the axes of geophilous Dicotyledons, for example many Umbellifers (Worsdell, 90), *Podophyllum* (Holm, 45).

When Professor Henslow suggested that all living Angiosperms were derived from a common dicotylar stock, he stood almost alone in that belief. Of late years the tide has begun to turn. More than one botanist has come to the same conclusion on independent grounds. But though their conclusion is the same, these observers are not agreed either with Professor Henslow or with each other on the method by which descendants from the primitive dicotylar stock became Monocotyledonous.

The latest contribution to the subject is that of Mr. A. W. Hill (39). Observations on an apparently monocotylar species of *Peperomia*, discovered by himself in a high Alpine situation on the Cordilleras, have led him to the conclusion that there are in fact two cotyledons present in the seedling. One is hypogeal and acts as a sucker; the other has assumed the appearance and functions of the foliage leaf. The species is—as might be expected from its habitat—very markedly geophilous in structure. Its seedlings seem to germinate in the spongy herbage of the locality. They are small, and their vascular tissue is so much reduced as to give them the appearance of aquatic seedlings, such as those of *Alisma*.

Mr. Hill's interpretation of the structure of these seedlings may be correct, but it is always necessary to be cautious in dealing with the homology of members much reduced from any cause. If, however, the seedlings of one species of a dicotyledonous genus have really become apparently monocotylar by the transformation of the second cotyledon into a foliage leaf, Mr. Hill has discovered a new method by which a dicotylar seedling may become monocotylar. He suggests that Monocotyledons may have been evolved in a similar way.

The vascular structure of seedling Monocotyledons does not support this view. One of the most striking features in it is the distinct vascular symmetry of the cotyledon and the first leaf (72, pp. 38–40). The cotyledon in the great majority of cases has no midrib. It is replaced either by a double bundle or by two single and quite distinct bundles. In the exceptional cases the apparently single midrib often shows its double character during the transition (*Zygadanus*), or is allied to—and probably derived from—forms with the more usual dual structure (*Veratrum*). The first leaf, on the other hand, always has a midrib, and commonly one pair at least of lateral bundles. This structure is repeated in all subsequent leaves. Schlickum describes three species of Monocotyledons in which the cotyledon closely resembles the first leaf. All three are aquatic plants: *Triglochin Barretieri*, *T. maritimum*, and *Alisma Plantago* (77, pp. 4–8). The vascular tissue is much reduced in all these plants owing to their aquatic

habit, and the cotyledon and first leaf alike have a single median bundle. The first leaf has two laterals in addition.

If the second cotyledon of the ancestor had really been transformed into the first foliage leaf, we should expect to find some traces of cotyledonary anatomy within it in forms not obviously reduced. But, on the contrary, the less reduced forms are those in which the anatomical contrast between cotyledon and first leaf is most conspicuous.

A third hypothesis is that both cotyledons of the ancestor united to form the single cotyledon of Monocotyledons. This view arises naturally from the comparative study of Monocotyledonous seedlings, and has already been discussed from the anatomical standpoint. But it is supported also by general considerations. I have already published the scheme founded on this hypothesis (72, 73). It suggests that the fusion of cotyledons which gave rise to the early Monocotyledons was an adaptation to a geophilous habit.

Two questions arise immediately out of that suggestion:

1. How could adaptation to a geophilous habit lead to fusion of the cotyledons?

2. Would it account for the peculiar stem-anatomy of Monocotyledons, and for some at least of the minor features which separate that class from Dicotyledons?

Before attempting to answer those questions, the characters of geophilous plants must be more fully considered.

Geophilous plants or geophytes are species which have their permanent axis underground. Their green organs are produced at the beginning of each growing season, and die down at the end of it. But this is a very wide definition. It would include all the forms known to gardeners as herbaceous perennials, besides the more specialized 'alpines' and 'bulbs'.

The more specialized geophytes are natives to climates in which a short season of growth is followed by a long period in which the conditions are unfavourable to vegetation. Such are alpine and arctic situations, where the summer may not last three months, and the ground is buried in snow for at least nine; or dry climates with periodic rains, as on the South African veldt, and in localities of the Mediterranean region. Geophytes form a large proportion of the flora in such climates. They are characterized by massive underground organs, which in the dead season represent the whole plant. At that epoch it consists of a squat axis, often much enlarged to serve as a storehouse for food, and of one or more buds, which will produce the aerial organs in the next season. Roots may or may not be present. Some geophytes (e. g. *Massonia pustulata*) have permanent roots which last over the dead season. Others (as many Orchids) have tuberous roots for aerial food-storage in place of tuberous stems. But the rule is that roots, like shoots, last over one growing season only, and die down at the end of it.

The linear leaves of Monocotyledons are particularly well adapted to sudden elongation in order to pierce the soil in the spring, when the ground is moist with the melting snow, or the first rains. The rapid production of flowers and leaves is a point of great importance to a geophyte. Every hour of the short season is precious—to the leaves for assimilation, to the flowers for ripening seed.

Another feature characteristic of a geophyte is the long interval which commonly elapses between the sowing of its seeds and their germination. This is a peculiarity only too familiar to gardeners who raise bulbous and alpine plants from seed. It is correlated with the very immature condition of the embryo in the ripe seed. Among many examples, I may quote the particulars as to the germination of *Ranunculus Ficaria* given by M. Sterckx (83). The embryo in the ripe seed of this plant is a small, spherical, undifferentiated mass of meristem attached to a short suspensor. Seeds sown immediately on ripening—that is, in May, 1896—did not germinate until the early spring of 1898 (pp. 42–3).[1] At the time of germination, cotyledon, plumule, and primary root were all indicated in the embryo, which had increased enormously in size.

This deliberate maturation of the embryo is correlated with the geophilous habit. For the plant has a struggle to get the seed ripened at all within its short growing season. To place the germ of the future plant in safety is the one essential: to provide it with food, and to protect it from the bad weather. This accomplished, the whole of the next growing season may well be devoted to the maturation of the embryo, while its germination is postponed to the third season. Hence the seeds of well-marked geophytes are commonly albuminous, and their embryos not only small but quite undifferentiated.

When germination at last takes place, the geophilous seedling makes but little show above ground. Sometimes, indeed, it remains below the surface during the first season after germination (*Arum maculatum*, *Veratrum nigrum*). But this is exceptional: as a rule at least one green part appears in the first year. It may be the cotyledon, as in *Fritillaria*, or the first leaf, as in *Crocus*. The fact that all Dicotyledons with one seed-leaf are geophilous has been mentioned already. So are those in which the cotyledons are partially united, as in *Podophyllum*, with the solitary exception of *Rhizophora* (72, pp. 73 and 77–8). It would seem, then, that the geophilous seedling observes a strict economy in its aerial organs during the first season of growth.

The explanation of this economy—already given elsewhere (72, pp. 80–1, and 78, p. 353)—may be shortly repeated here. The seedling has to face the return of bad weather at the end of a short growing period. If it is not

[1] Two seeds among a large number are stated by M. Sterckx (83) to have germinated in 1897. Dr. Schmid in South Germany (78), and Mr. McDonald in Lancashire (59), found that seeds sown early out of doors commonly germinate in the following year. No doubt this discrepancy depends on a difference of climate.

well sheltered from adverse conditions by the end of its first season, it will not survive to the next. Geophilous seedlings take shelter in the earth. Hence their underground organs are of the first importance. A bud must be formed, protected both by its coverings and by the soil in which it is sunk. It must be provided with food on which to start growth when the genial weather returns. The provision of such a bud in the limited period allowed to the seedling taxes its resources. Only a very small aerial shoot can be afforded in addition, even though in the end the green parts more than pay their way.

The first question proposed on page 179 has been already answered. The geophilous habit is correlated with partial fusion of the two cotyledons in some Dicotyledons, and with a single cotyledon in others. Supposing one branch of descendants from the Primitive Angiosperms to become geophilous, the green parts of their seedlings would inevitably be reduced in the early seasons of growth. In the first season this reduction might well take the form of partial fusion of both cotyledons, leading to complete fusion.

The second question can perhaps be answered most graphically by describing a concrete instance. The seedling of *Podophyllum peltatum* has been fully described by Mr. Holm (45). Its cotyledons are united into a long tube: the blades alone are distinct. We have already seen that its vascular symmetry recalls that of a Monocotyledon (p. 173, and Figs. 19–21). The anatomy of the erect stem in the mature plant is markedly Monocotyledonous (Holm, 45, and Solereder, 81, p. 52). The radical leaves are net-veined, but their petioles are expanded at the base into a sheath from which many traces enter the subterranean stem-bud. Mr. Holm has shown how the scattered disposition of bundles in the stem follows from this arrangement. The traces in the erect stem have lost their cambium—they are closed like those of a Monocotyledon—but cambium is found in the bundles of the rhizome. These are arranged in a single circle, but without intrafascicular cambium.

Podophyllum peltatum grows in swampy woods in the Southern States of North America. Its squat underground axis, packed with starch, though not expanded into a tuber, is a geophilous character, probably developed under climatic conditions of greater severity than those of its present habitat. There are bulbous plants in English woods—the bluebell, *Scilla festalis*, Salisb., for example—which are above the ground in spring and early summer only. At this season such plants are sheltered by the leafless trees without losing much light. But though their geophilous habit enables these species to take advantage of the woodland stations which they now occupy, it was probably acquired elsewhere: inherited perhaps from ancestors living under more stringent conditions. In the case of *Podophyllum* this is the more likely as the only other species of the genus, *P. Emodi*, is Himalayan.

In addition to the Monocotyledonous characters already mentioned, *Podophyllum peltatum* has a 6-partite perianth.

The partial fusion of the cotyledons and the anatomy of the erect stem in *P. peltatum* can be fairly interpreted as adaptations to a geophilous habit. A Ranal stock with similar characters, if exposed to more stringent conditions of the same kind, might easily go on to complete fusion of the cotyledons. It might not develop the lateral underground shoots which enable *P. peltatum* to spread in its swampy habitat, but the squat underground axis would probably be enlarged into a tuber with a fresh crop of root, each season. The aerial shoots would certainly be reduced in size. Such a form would not be far removed from a true Monocotyledon.

Several objections to the geophilous origin of Monocotyledons which have been urged, or have occurred to me independently, may be considered here.

It has been suggested that so large a class as Monocotyledons, and one so varied in habit, is not likely to spring from a race of plants so highly specialized to peculiar conditions. A plant may, however, be distinctly geophilous without being so completely adapted to its immediate surroundings that its descendants can never change their habit. The geophilous primary axis of *Podophyllum peltatum* has not prevented it from acquiring the rhizome suited to a swampy station. Indeed, a geophyte which has not developed a pronounced tuber or bulb can readily adapt itself to an aquatic life. Its underground stem roots first in the muddy banks, and thence advances into the bed of a pond or stream. In the course of generations its aerial shoots become more and more adapted to submersion. Many comparatively ancient Monocotyledons, such as *Alisma*, have perhaps escaped extinction by taking possession of such situations.

Climbers, again, often possess massive underground stems from which aerial shoots are sent up every year (*Bryonia*, *Tamus*).

The geophilous structure of the stem is certainly unfavourable to the evolution of trees. This is perhaps the line in which Monocotyledons are least successful. No Monocotyledonous tree can compete with Dicotyledons outside the tropics. Within them, Palms have contrived to overcome the disadvantages of an essentially geophilous structure. The early growth of Palms, the slow formation of their axis in their early years by the addition of one disk after another to a squat subterranean stem, the close connexion of the early leaves with their special roots, all point clearly to a geophilous period in the history of the race (cf. Sargant, 73, p. 344).

The conditions of life which encourage geophilous characters are very local at the present time. Alpine summits are necessarily isolated: dry climates with periodic rains occur in limited regions scattered all over the globe. It is certainly difficult to conceive how a race formed in any one

of these localities should reach the others and spread all over the globe as Monocotyledons have done. But conditions may have been more favourable in an earlier geological epoch. During the glacial periods the winter in Europe and Northern Asia was much longer and colder than at present: the summer shorter and warmer. The Alpine flora of this continent is supposed to represent the survivors of a flora which spread all over it during these epochs. On such a stage as this the great drama of the evolution of Monocotyledons might well be played with success.

The geophilous habit does not, so far as I can see, explain the ternate floral symmetry of Monocotyledons. Whorls of three and six parts are not uncommon in the flowers of the Ranales. It is possible that they are inherited from an ancestor which this alliance has in common with Monocotyledons. I agree with Messrs. Arber and Parkin in believing that the stamens and carpels of the Primitive Angiosperm were indefinite in number and spiral in arrangement. The perianth may, however, have been in whorls of three or six.

The hypotheses which have been considered with regard to the number of cotyledons in the Primitive Angiosperm and their morphological nature have led to two phylogenetic schemes only which attempt to explain the evolution of Monocotyledons and Dicotyledons from a common ancestor. The first is that of Professor Henslow which derives Monocotyledons from a dicotylar race by suppression of one cotyledon and adaptation to an aquatic habit. The second is the fusion hypothesis. The two cotyledons of Primitive Angiosperms have united to form the single member in Monocotyledons. This union, as well as the peculiarities of stem anatomy, and many of the minor characters in Monocotyledons, are due to adaptation to a geophilous habit.

Both schemes, it will be observed, give the Primitive Angiosperm two cotyledons and the stem anatomy of a Dicotyledon. Most of the minor characters which distinguish Monocotyledons are also treated in both schemes as departures from the primitive type. It is probable, therefore, that the Primitive Angiosperm resembled Dicotyledons much more nearly than Monocotyledons in their general features, as well as in stem anatomy and the possession of two cotyledons.

QUARRY HILL, REIGATE.
Jan. 20, 1908.

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Bio-histological notes on some new Rhodesian species of Fuirena, Hesperantha, and Justicia.

BY

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With Plates XI and XII and ten Figures in the Text

IN a former paper[1] some points of biological interest in connexion with the above plants collected by the author in Southern Rhodesia were briefly touched upon. The results of further investigation are now given in greater detail, and show very marked adaptation to physiological conditions, which in the case of *Fuirena Oedipus* and *Justicia elegantula* have resulted in types of peculiar specialization quite outside the known limits of their respective families.

On the high Matabeleland plateau of Southern Rhodesia the rainfall varies from 17 to 30 inches, occurring chiefly in the form of thundershowers, and it is entirely limited to the summer or hottest months of the year, when evaporation is greatest. The general altitude is 3,000–4,500 feet, with a mean annual temperature of 65° to 70° and five absolutely rainless months in the year. In a tropical country of this description the rainfall is sufficient to support a stunted growth of trees, many shrubs, and herbaceous plants. These meet the extreme conditions to which they are exposed rather by husbanding the general underground water supply in the soil than by elaborating individual forms of water reservoirs. Water storage tissue seems more characteristic of regions of slight intermittent rain and short periods of surface moisture.

The long seasonal winter drought, with cold nights and hot days, is met, in the case of trees, by the throwing off of leaves and by a great variety of cork protection. The efficiency of this enforced quiescence is proved by the fact that the severe frosts which sometimes occur at this season leave the native vegetation untouched, though they are often injurious to introduced trees and shrubs.

Herbaceous plants have evolved most massive underground root systems, and if they do not die down every year they are cut back by the veld fires. The aerial parts are characterized either by a flat spreading habit

[1] Gibbs, A Contribution to the Botany of Southern Rhodesia. (Journ. Linn. Soc., xxxvii, 1906, pp. 426–94, Pls. XVII–XX.)

[Annals of Botany, Vol. XXII. No. LXXXVI. April, 1908.]

or by an extreme leaf development, or the specialization of vegetative shoots which succeed the earlier flowering ones. These secondary vegetative shoots, as well as many herbaceous and other plants with soft woody stems, show a decumbent tendency, ultimately spreading on the ground. Striking organographical deviations to meet physiological conditions are conspicuous by their absence, and this fact is no doubt due to the sufficiency of underground moisture which can be drawn upon as required. The limitation of that supply, however, is shown by the strict economy exercised in its distribution. The characteristic spacing of trees, already referred to [1], is reproduced in the growth of herbaceous plants, and may probably be referred to this factor. Each plant whilst exhausting the water supply in its own vicinity tends to crowd out other competitors by extending its shade area, and thus exercises, possibly as a secondary result, a retarding influence on evaporation.

This bare sufficiency of soil moisture is apparent in the general beginning of plant life in September and October, before the rains. The rigid economy necessitated by the limit of that supply is seen in the tendency of trees and shrubs to flower before the leaves expand, and this habit is further modified in herbaceous plants by the development of specialized flowering shoots. In this way the whole energy of the plants is centred on vegetative activity only during the short season favourable to growth.

The bulbous annual tender herbaceous plants are apparently restricted to the summer or rainy season. The few species of *Crinum*, *Buphane*, *Albuca*, *Urginea* and *Gladiolus* which signal the early spring invariably send up the flowering scapes first, even the fruit maturing before the leaves appear; but in the case of bulbs of *Albuca caudata* Jacq. and corms of *Gladiolus Melleri* [2] brought back by Baker this habit has proved to be due to biological conditions. Under favourable cultivation both flowering scape and leaves have been simultaneously developed. The same remarks apply to trees and shrubs found growing under varying conditions in their natural surroundings.[3] The *Fuirena* and *Justicia* species here described would thus prove exceptional to the general rule, being characterized by very distinct organographical modification which takes the form of starch storage tissue, in the former a portion of the stem, and in the latter special leaves, being involved in its elaboration. In both examples, however, this specialization is not to meet the long physiological drought conditions of widely different edaphic character (*Fuirena Oedipus* being a hygrophilous and *Justicia elegantula* a veld-type) to which each plant is exposed, but to ensure sufficient reserve food supplies to promote a rapid spring growth, with the least expenditure of energy.

[1] Gibbs, l. c.

[2] This case is particularly interesting, as the corms were given to Dr. Bolus at Cape Town and grown out of doors. The first time the plant flowered, the foliage did not come up till two months later, but the second year both scape and leaves came up together.

[3] Gibbs, l. c.

FUIRENA OEDIPUS C. B. Clarke.

The aerial shoots of this species are about 20–40 cm. high (Pl. XI, Fig. 1), and these arise at more or less regular intervals on the horizontal rhizome. The stem is four-angled, but the basal node of each aerial shoot is modified in the form of a round sessile pseudo-bulb with contracted base. These swollen internodes when collected were white and gleaming with a smooth surface, and are very apparent as they burst through the brown membranous scale-leaf of the node. The basal nodes are most developed in the flowering shoots of the current year (Fig. 2, *b.i.*) where they are globose in shape. In the vegetative shoots they are more elongated (Fig. 1); while in the dead shoots of the previous year, having given up all their reserve food material, they have shrunk both to the quadrangular shape and to the usual proportion of the stem (Fig. 1, *d.s.*). The plant was found at the Victoria Falls in September, growing in what, in a previous paper[1], is described as the bog edge of the so-called Rain Forest. This bog edge consists of a zone of hygrophilous plants, varying in width and plant association, both apparently being determined by the intensity of the spray-fall from the cataract on the opposite side, during and after the rainy season, when the Zambesi is in flood. The river then falls in one sheet of water over its whole width, and the spray, which has been measured by theodolite to reach the height of 3,000 feet, is continuous and may be described as perpetual rain. When this plant was in flower, the river was almost at its lowest, reduced to about four separate falls. It was found growing almost opposite to the Devil's Cataract, where only grasses and sedges formed a tangled mass. The spray at that season falls intermittently as a fine mist, according to the direction of the prevailing breezes. The foliage was sometimes dry, but generally a dew-like moisture rested on the leaves, notwithstanding the continuous sunshine above.

As might be expected from such surroundings, the plant is very hygrophilous in habit, the upper leaves showing long and broad laminae, while the scale leaves of the lower stem-internodes, and of the rhizome, are quite membranous in texture and not persistent. These facts, considered with the histological examination, agree with the results of other workers on the Gramineae and Cyperaceae, where biological conditions in relation to habitat have been taken into account[2]. Unfortunately investigation was limited to the type specimen, which increased the usual difficulties in dealing with herbarium material.

HISTOLOGY

Rhizome. The rhizome is small, about 2 mm. in diameter throughout, with short internodes. It is covered with brown scale leaves, in the axils

[1] Gibbs, l. c.

[2] Spinner, L'anatomie foliaire des *Carex* suisses, 1903.

of which the aerial shoots arise from a bifurcation of the rhizome. The branching is sympodial, and several aerial stems are given off in one season, both flowering and vegetative (Pl. XI, Fig. 1). The rhizome consists of a solid central cylinder (Pl. XII, Fig. 8) composed of many fibro-vascular bundles embedded in fundamental tissue: it is bounded by an endodermis accompanied by several layers of sclerotic cells (Fig. 8, *e. sch.*). A very broad cortex of unmodified parenchyma succeeds the endodermis and the sclerotic zone. Of this the peripheral portion is composed of larger cells, is not aerenchymatous, and is reinforced by hypodermal ribs and small isolated groups of more centrally placed sclerotic tissue (Fig. 8, *sch.*). The epidermis is slightly cuticularized, and tannin is abundant through the rhizome where starch occurs in the cortex, but not in the central cylinder. The cortex in the Cyperaceae, according to Plowman [1], is highly susceptible to environment and varies in the same species. Duval-Jouve [2] observes that in hot and damp localities in Algeria the cortical zone is soon destroyed, but persists longer in dry and sweet ('frais') places. He mentions this fact against Guillard's [3] theory that the size of the cortical envelope of roots and rhizomes depends on the humidity of the soil and not on a special organization. The present case would seem to support the latter author.

In the central cylinder the fibro-vascular bundles come under Plowman's [1] 'amphivasal' type, the bundles being concentric with the xylem distributed round the phloem (Pl. XII, Fig. 8, *a.v.b.*). The bundles are very numerous, especially near the periphery of the central cylinder. The central strands are larger, characterized by a large centripetal mass of sclerenchyma showing protoxylem lacunae. The section Plowman [1] describes and figures for *Scirpus cyperinus* Kunth might pass for *Fuirena Oedipus*, with the exception of the sclerotic ring round the endodermis, which, according to him, is characteristic of *Fuirena*.

Aerial stem. In this species the stem is quadrangular, as is also the case in *F. umbellata* Rottb.,[4] but otherwise this seems to be a very uncommon feature in the Cyperaceae, in which the stems are usually round or triangular. The first three or four internodes of the stem are very short and covered by membranous scale leaves, light brown in colour. A cross section through the third internode from the rhizome shows a typical hygrophilous structure (Pl. XII, Fig. 11) with large medullary air-spaces of schizogenous origin, separated by delicate plates of parenchyma which accompany the medullary bundles. The cortical bundles are arranged

[1] Plowman, Comparative Anatomy and Phylogeny of the Cyperaceae (Annals of Botany, xx, 1906, pp. 1–33, Pls. I–XI).

[2] Duval-Jouve, Étude histotaxique des *Cyperus* de France (Mémoires de l'Acad. des Sci. et des Lett. de Montpellier, viii, 1874, pp. 347–412, Pls. XIX–XXII).

[3] Guillard, Bull. Soc. Bot. de France, xvi. 420.

[4] Rickli, Beiträge zur vergl. Anat. d. Cyperaceen, m. besonderer Berücksichtigung d. inneren Parenchymscheide (Pringsh. Jahrb. f. wiss. Bot., xxvii, 1895, pp. 485–580, Pls. XVIII–XIX).

regularly on the periphery, each accompanied by a hypodermal strand of fibres alternating with large lysigenous air-spaces (Fig. 11). Tannin sacs are present, and starch is massed in the two or three layers of parenchyma (Fig. 11, *t.s. st.*) which occur between the phloem and the hypodermal strands of fibres accompanying the cortical bundles. No starch was found in the sheath of colourless cells which surrounds each bundle. In this section the epidermis is without stomata, is thinly cuticularized, and its cells are smaller over the hypodermal ribs.

Basal internode. As has been already described, the lowest internode of the aerial stem is curiously modified for starch-storage purposes, and its form is due to a proliferation of the cortical tissue of the internode. The cross section is circular (Pl. XII, Fig. 9), the lysigenous air-spaces of the aerial stem being quite suppressed, though the sclerenchymatous ribs are still present, and the cells of the epidermis are smaller where they pass over them. The cortical bundles are pushed more towards the centre, showing that it is the starch-containing layers of parenchyma already indicated in the aerial stem (Pl. XII, Fig. 11, *st.*) which are responsible for the reserve storage tissue of the pseudo-bulb formation. Small schizogenous air-spaces occur freely in the cortex, and increase in size towards the medulla (Fig. 9), which is identical in structure with that of the unmodified portion of the stem (Fig. 11). All the cells, including those of the thin medullary plates of parenchyma, are densely packed with starch, resulting in a structure of corm-like consistency, the rigidity of which is due entirely to its stores of reserve material; the mechanical thickening, notwithstanding the great increase of surface, being no greater than in the upper portion of the stem. Tannin sacs are very numerous in the cortical region.

In longitudinal section (Pl. XII, Fig. 10) the subtending leaf-trace bundles are seen to insert themselves on the cortical bundles of the stem at each node (Fig. 10, *l.t.b.*) essentially as figured by Plowman[1] for *Dulichium arundinaceum* Brit., and the arrangement is similar in the basal node, in which the reserve food tissue is strictly limited to the proliferation of the cortical parenchyma.

Examples of special organogenic modifications for the storage of reserve food material are of exceptional occurrence in the Cyperaceae, and, as far as investigation goes up to the present, are entirely limited to the rhizome. Holme[2] has recorded for *Fuirena squarrosa* Michx. a tuberous development of one of the internodes of the rhizome, which takes the form of a shoot with the growing point arrested, the tissue composing it being packed with starch. He cites this case as being very rare in the genus, comparing it with the tuberous rhizome of *Cyperus esculentus* Linn. and *C. Rhymatodes* Muhl. described by Duval-Jouve[3] as being formed from

[1] Plowman, l. c.

[2] Holme, Studies in the Cyperaceae (Am. Journ. Sci., 1897, pp. 13-25, Pls. 1-2). [3] Duval-Jouve, l.c.

several internodes which, at the point where the rhizome bifurcates in the formation of the aerial stem, instead of elongating like the others, remain short and swell to a considerable size, the rest of the rhizome being slender and wiry. In grasses, tuberous swellings involving the lowest internodes of the culm and foliage-shoots (Knollen-Gräser) or the base of the leaf-sheaths (Zwiebel-Gräser) are common. According to Hackel[1] these are characteristic of species inhabiting regions of periodical drought, where they serve as water reservoirs, he having never found any reserve material in them, at whatever season examined.

The late Mr. C. B. Clarke, whose knowledge of the Cyperaceae was unrivalled, was much struck by this plant, to which he gave such a peculiarly appropriate specific designation. A similar modification was unknown to him in the order.

The leaf. In this species the lamina is 6–7 mm. broad, 15–18 cm. long, with five prominent nerves on the dorsal side, corresponding to the same number of grooves on the ventral surface, which is covered with unicellular hairs, such as, according to Rickli[2], are typical of *Fuirena.*

The structure of the leaf is very interesting owing to the extreme differentiation of the epidermis on the ventral surface, the cells composing it being thin-walled and enormous in size. (Pl. XII, Fig. 12, *v.e.*)

As the genus *Fuirena* has been hardly touched upon in work on other families in the order, sections were cut of the leaves of two other species collected in the same season in the Matoppo Hills, but found growing under different conditions and showing a more xerophytic habit. For purposes of comparison these are now described.

Fuirena stricta Steud. A plant of very wide distribution throughout tropical Africa and the Mascarene Islands. It was growing in a bog, in probably a stagnant and acid substratum. The stem is triangular, with very long internodes almost covered with nearly equally long leaf-sheaths, the laminae of which are very much reduced, being narrow and very short. The scale leaves of the rhizome and the lower stem-internodes are membranous. In this case the cells of the epidermis on the ventral surface of the leaves are large. *Fuirena subdigitata* C. B. Clarke[3] shows a more hygrophilous habit, but the structure of the leaves is very xerophytic in type. The laminae are considerably longer than the encircling leaf-sheaths. The stem is triangular, with long internodes not entirely covered by the leaf-sheaths. This plant was growing on damp sand-banks in a stream, the roots therefore in moisture, while the aerial shoots would be exposed to powerful illumination. The scale leaves are in this case very hard and persistent, possibly to withstand the effects of inundation in the rainy season.

[1] Hackel, Ueber einige Eigenthümlichkeiten der Gräser trockener Klimate (Verhandl. Zool.-Bot. Ges. Wien, 1890, pp. 125–136).

[2] Rickli, l. c.

[3] Gibbs, l. c., p. 477.

The epidermis. Cuticularization is not very pronounced, showing little reaction to iodine or sulphuric acid, but the area of the cuticle is increased by papillary outgrowths of hammer-headed form (Pl. XII, Fig. 14). This 'granulation,' as Rickli[1] calls it, has been described by various authors and considered typical of hygrophilous species in the order. Rickli found that it varied in the same species and was limited to the neighbourhood of the stomata, and, as these lie in channels, water and rain could follow the depressions and so swamp the apertures. The granulation, he surmised, would hold the water and so prevent the temporary cessation of stomatic function. Possibly it might have that effect and in some species may be more pronounced in the vicinity of the stomata, but in *F. Oedipus*, where it is particularly well developed, and in the other species examined, it occurred over the whole surface of the leaf, which on the vertical side is devoid of stomata. In the former the cuticle of the stem, with the exception of the basal node, shows the same peculiarity.

The stomata consist of four cells of the usual type limited to the Gramineae and Cyperaceae (Pl. XII, Fig. 14 *a*), the mechanical structure of which has been very thoroughly worked out by Schwendener.[2] In considering the position of the stomata he states that in the representatives of steppe and desert flora they are sunk, but this condition is also found in the inhabitants of boggy places. The latter point is brought out in rather a striking manner in the present case, the stomata showing different positions in each of the three species collected, though all might be ascribed to the above habitat. In *F. Oedipus*, where the aerial stem is usually bathed in moisture, the stomata are raised above the surface of the epidermis (Pl. XII, Fig. 12), possibly ensuring more rapid transpiration, which cannot go on where the surrounding atmosphere is saturated with water-vapour, but perhaps also the position may serve to check the lodging of films of water across the orifices of the stomata. In *F. sub-digitata*, where the rhizome alone is in perpetual moisture and the aerial shoots are exposed to full light and heat conditions, the stomata are not only sunk, but the cuticle of the guard-cell is produced into four papillae which project across the opening, almost covering it. This is precisely what Volkens[3] has described for some Carices growing in similar situations, notably *C. panicea* Linn. The flooding during the rains to which *F. sub-digitata* must be subjected may also account for this development of the stomata, which in *F. stricta* are only slightly sunk and not provided with protective covering, though found growing in a bog medium liable to desiccation.

The cells of the epidermis throughout the Cyperaceae show distinct

[1] Rickli, l. c.

[2] Schwendener, Die Spaltöffnungen der Gramineen u. Cyperaceen (Sitzungsb. K. Akad. Berlin, 1, 1889, pp. 65–78, Pl. I).

[3] Volkens, Zur Kenntniss der Beziehungen zwischen Standort u. anat. Bau der Vegetationsorgane (Jahrb. Bot. Gart. Berlin, iii, 1884, pp. 1–46, Pl. I).

differentiation on each surface of the leaf, those on the dorsal surface being much smaller than those on the ventral or upper side. The contents of all the cells are colourless, and only the outer walls are thickened, the radial and inner tangential walls being of an extremely delicate texture.

On the dorsal surface they are smaller over the hypodermal ribs (Pl. XII, Figs. 12 and 13), where the development of the hard fibrous cells seems to arrest their radial expansion, which is shown in a transverse section through a developing leaf of *C. stellulata* Good (Fig. 15). In the youngest stage obtainable, the sclerenchymatous cells not having yet differentiated out, those of the epidermis are still more or less equal in diameter. The peculiar cone-like base limited to the most radial of these cells noted by Duval-Jouve[1] in *F. pubescens* Poir. was not seen in the species examined. The ventral epidermis is marked by enormous thin-walled cells with colourless contents (Pl. XII, Figs. 12 and 13). The outer walls alone are cuticularized together with the unicellular hairs which are scattered over its surface, the radial and inner tangential walls are very delicate in texture. These cells in the vicinity of the vascular strands are radially elongated, and there is always an intimate connexion with the bundle-sheath of delicate thin-walled cells (Figs. 12 and 13). This connexion is continuous with the dorsal epidermis by means of small colourless cells passing up both sides of the sclerenchymatous ribs which accompany the bundles on that side.

The large cells which occur in groups in the epidermis of grasses, and throughout the Cyperaceae, characterize the entire ventral epidermis of the leaf in varying degrees of differentiation, and have been described as 'bulbiformes' by Duval-Jouve, who observed that stomata were always absent on a 'bulbiforme' epidermis. Tschirch[2], who looked upon them as characteristic of steppe and meadow grasses, gave them the name of 'Gelenkzellen', as he considered they controlled the folding and unfolding of the leaves of grasses by their turgescence or loss of water. Volkens[3] states that he has never observed the folding up of the lamina of a grass leaf, but always a windbag (Blasebalg) action, as the lamina increases or decreases in width according to the turgescence (Wasserzufuhr) of these cells, which proves they function as a water reserve. He therefore rechristened them 'Wasserzellen'.

Rickli[4], noticing the delicate radial and inner tangential walls of all the epidermal cells, looks upon the whole organization as a water-jacket, and there can be no doubt that this interpretation is correct. This is shown by the connexion, between the two surfaces, with the bundle-sheaths, as already pointed out, by the delicate inner walls, and by the limitation of the stomata

[1] Duval-Jouve, Sur une forme de cellules épidermiques qui paraissent propres aux Cypéracées (Bull. Soc. Bot. France, xx, 1873, pp. 91–95).

[2] Tschirch, Beiträge zu der Anatomie u. dem Einrollungsmechanismus einiger Grasblätter (Pringsh. Jahrb., xiii, 1882, pp. 544–68, Pls. XVI–XVIII).

[3] Volkens, Flora der ägyptisch-arabischen Wüste, 1887. [4] Rickli, l. c.

to the dorsal side, which allows of a larger size of the water-jacket cells on the ventral or exposed surface. This fact, coupled with the entire absence of extreme cuticularization and hairy covering in the order, shows the adequacy of this simple protection.

As Rickli [1] expressly dwells on the delicate and radially stretched walls of the ventral epidermis of *Fuirena scirpoidea* Mich., *F. repens* Kth., it was suggested that the development of this tissue, so conspicuous in *F. Oedipus*, might be a special character in the genus, but examination of the other species available did not bear out this hypothesis.

In *F. sub-digitata* these cells are about the same size and shape as in many species of *Scirpus*, *Cyperus*, and *Carex*. In *F. stricta* they are larger and radially elongated, but not so conspicuously different as in *F. Oedipus*. In both species, however, the connexion with the bundle-sheath is very well marked, especially on the dorsal side, where the hypodermal ribs are better developed than in *F. Oedipus*, and the connecting colourless cells, which break away from the sheath and run up each side of the fibrous groups, are more numerous and larger in size.

The larger area of leaf surface in *F. Oedipus*, coupled with the organization of the stomata for rapid transpiration, no doubt necessitates the reinforcement of the water-jacket on the exposed ventral surface. It is the only organization for the protection of the palisade tissue, and the very marked increase in the size and volume of the cells proves what a very efficient as well as plastic organization such an uninterrupted water reservoir must be.

Palisade and mesophyll tissue. The former is well differentiated (Pl. XII, Fig. 12), a fact, according to Plowman [2], rare in the Cyperaceae, but also seen in *F. stricta* and *F. sub-digitata*. It is composed of one layer and shows a decided convergence towards the bundle-sheath (Figs. 12–13). The *mesophyll* has a tendency to break down, forming the characteristic large lysigenous air-canals, which alternate with the bundles and are so typical of the order. Traces of diaphragms occur across these air-spaces in the mature leaf, but are composed of plates of mesophyll and show no approach to stellate form. In *Carex stellulata* these air-spaces were traced back, and it was only in the very youngest stage that the leaf tissue was found entire (Pl. XII, Fig. 15), and even then the area to be destroyed is marked by the larger intercellular spaces, resulting in a looser arrangement of the cells. In *F. sub-digitata* these canals are not present. They are replaced by large thin-walled cells with colourless contents, which show lateral connexions with the bundle-sheaths, and they no doubt reinforce the water-storage tissue of the leaf. The breaking down of parenchymatous tissue in this way may possibly be connected with rapidity of transpiration. In the stem the cortical parenchyma between the peripheral bundles is broken down, and in the leaves the mesophyll tissues are similarly affected.

[1] Rickli, l. c. [2] Plowman, l. c.

Areschoug[1] describes transpiratory parenchyma as distinguished by large lacunae, and where rapid transpiration is necessary the palisade tissue is reduced and the mesophyll increased. When transpiration is reduced, a system of water storage or palisade is elaborated. In *Fuirena sub-digitata* this result is obtained, and the air canals are replaced, through the whole length of the leaf, by water-storage tissue associated with stomata that are not only sunk but have their apertures partially covered over.

Vascular tissue. The vascular bundles are collateral. The phloem, directed towards the dorsal surface, is composed of small thin-walled cells with well-marked companion cells. A certain amount of starch is present in the phloem. The two vessels are always accompanied by a protoxylem lacuna and lignified parenchyma (Pl. XII, Fig. 12, *px. l.* and *l.p.*).

Bundle-sheaths. A sheath of large thin-walled parenchymatous cells, colourless in contents, surrounds each bundle (Fig. 12, *w.s.*), the water-sheath noted by Rickli[2] as occurring in many Cyperaceae. The inner 'Parenchymscheide' which the same author figures as characteristic of the Chlorocyperaceae, including *Fuirena*, was not observed in the species examined, though in *Fuirena stricta* a very well-marked sclerotic sheath of single cells surrounds each bundle inside the water-sheath. No starch was traceable in the cells of the latter, towards which the palisade cells show a marked convergence. The connexion with the epidermal water-cells provides an organization which surrounds all the vital parts of the leaf, not only reducing the temperature, but ensuring turgescence by eliminating transpiration on the most exposed surface. Thus protection against transpiration is effected by a very simple and, as we have seen, plastic organization, while other plants have to meet the difficulty by elaborate contrivances, involving much greater specialisation of structure, such as extreme cuticularization or collenchymatous thickening of the epidermal walls, secretion of mucilage, or hairy coverings.

The anatomical characters of the three *Fuirenas* examined are thus seen to be considerably modified by their environment, though from a general point of view their organization is on the well-marked developmental lines common to all the genera of the order. In *Fuirena Oedipus* the plant provides for a short and rapid growth-period by laying down starch reserves in the basal node of the stem. This is an advance in organization to storage in the rhizome, as it not only ensures the supply being further removed from the constantly wet substratum in which roots and rhizome lie, but also provides a short cut when the plant starts into growth, as the starch has not to be transferred from the rhizome to the aerial tissues. In the structure of the leaves, the hygrophilous habit is shown in the breadth and length of the lamina, also

[1] Areschoug, Der Einfluss des Klimas auf die Organisation der Pflanzen, insbesondere auf die anatomische Struktur der Blattorgane (Engler's Bot. Jahrb., 11, 1882, pp. 511–526).

[2] Rickli, l. c.

in the unprotected stomata, which suggest a shade habit. The hereditary organization of the plant, which enables it to form the epidermal water-jacket and dorsal stomata, has proved sufficiently plastic to meet altered conditions. Aerial exposure to intense illumination and intermittent dryness is made possible by materially increasing the size and volume of the water-cells on the exposed surface of the leaf, and a satisfactory adaptation to present environment is thus secured.

Where the aerial stem bears rather broad leaves with moderately well-developed lamina and is exposed to constant xerophytic conditions, we find the modifications more complete, and in *F. sub-digitata* we get the protected and sunk stomata and an increase of water-tissue which replaces the air-canals. In *F. stricta* on the contrary, where the leaves are fewer and the laminae very much reduced, beyond a slight sinking of the stomata and the reinforcing of the water-sheath by a strong sclerotic one, the hereditary organization of water-jacket and papillose cuticle has proved sufficient.

HESPERANTHA MATOPENSIS Gibbs.

This Iridaceous species was found growing amongst grass on the sandy banks of a stream, which had cut a channel through the sand a metre below the level of the veld. It was in flower and leaf in September, before any rains had fallen, after a period of five months' drought. The flowering scape was on a level with the surrounding yellow grass haulm, which the flowers, that only opened at night, rather resembled in their light straw-brown colour. Towards evening the perianth segments would reflect, exposing the style and stamens, and a delicate perfume was emitted.

The interest of the plant centres in the extraordinary development of the tunics in comparison with the minute size of the corm to which they act as a protective covering. The corms with the tunics are from 12 to 15 cm. long and 14–18 cm. broad; but the actual corm itself only measured 5 mm. in length and 8 mm. in breadth, the difference in size being accounted for by the old scale-leaves of each year's successive growth persisting as rings one inside the other, round the corm.

The morphological development of these scale-leaves, of which one only is produced every year, is very interesting and obtains throughout this genus and the allied genera of *Lapeyrousia* and *Geissorhiza* in varying degrees of complexity, but *Hesperantha matopensis*, as compared with material in the British Museum and at Kew, apparently shows the most extreme form.

The scale-leaves are symmetrical in form, flattened at the base, dark brown in colour, and of a hard, smooth consistency. They are split about a third of their length into longitudinal segments with laciniate margins (Text-fig. 1, *t*) due to a folding of the scale-leaf tissue, the split occurring up the centre of the fold. At the base round the flattened portion of the

scale a horizontal fold occurs, and there the young corms break through, the two margins being gradually pushed apart by the growth of the corm-lets (Text-fig. 2, *yg.c.*), leaving the same laciniate margins as on the longitudinal segments. The peltate base of the tunic then separates off as a round scale (Text-figs. 2 and 3, *p.b.*) while the upper portion remains as a persistent ring round the neck of the corm (Text-fig. 1, *t.*). In Text-fig. 4, where the base of last year's tunic has been removed, the young corm in its axil is seen resting against the base of this year's scale-leaf. In specimens of *H. matopensis* collected three to four of these rings only were

FIG. 1.

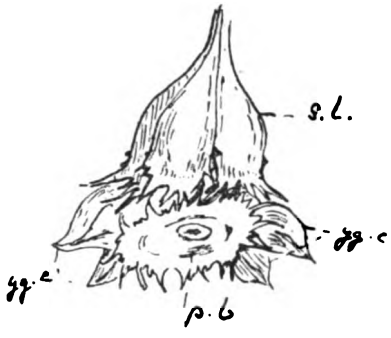


FIG. 2.

FIG. 3.

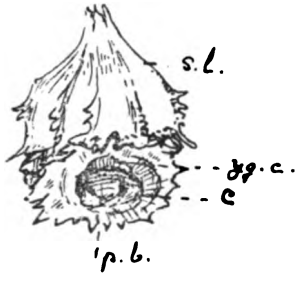


FIG. 4.

Hesperantha matopensis.

counted, but in a specimen of *H. falcata* Ker. Gawl. (Brit. Mus.) as many as eight scales were seen, occurring one above the other.

The tissue of the scale-leaf is composed of several layers of parenchyma, the inside layers of which lignify, while the outer ones remain membranous and form a white skin on both surfaces. The laciniation of the margins is caused by the splitting of the scale-tissue (a section through one of these teeth as shown in Pl. XII, Fig. 17, *l.p.*). Faint vascular strands consisting of one or two vessels run longitudinally down the scales, but are of no account in the development; the dark-brown colour is due to some form of colouring matter, not tannin, which only dissolves out after a week's soak-

ing in Eau de Javelle. In a longitudinal section through a corm (Fig. 7) it will be seen that the folding of the scale-tissue which results in the longitudinal segments of the mature tunic is caused, like the basal scale, by the shape of the corm itself, which is furrowed; it is these depressions which evidently induce the proliferation of tissue over their areas. The same proliferation occurs round the flattened base of the corm (Text-fig. 5, *h f.*) Text-fig. 6 shows the scale of a very young cormlet, still attached to the parent corm. In this stage the scale is entire, light yellow in colour, and membranous in consistency. The peltate base is indicated, following the shape of the cormlet, and the ridges already present in the latter have begun to form.

In comparing the tunics of *H. matopensis* with other species in the genus, a series is shown in the degrees of laciniation and the splitting of the margins, also in the longitudinal folding and the formation of the basal scale.

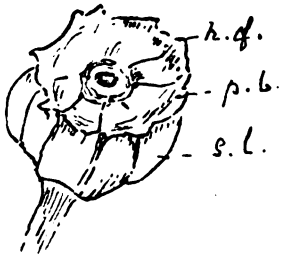


FIG. 5.

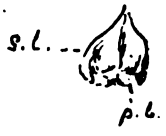


FIG. 6.

FIG. 7.

Hesperantha matopensis.

In *H. pilosa* Ker. Gawl., *Baurii* Baker, *candida* Baker, *lactea* Baker, and *falcata* there is no apparent peltate base to the tunics, the corm itself not being flattened, the tunics accordingly separate off at the actual base of the latter and the old persisting tunics consequently fit one above the other, each succeeding one being a little longer than the one which preceded it.

The tunics are also smooth with no foldings, following the round shape of the corm. In *H. radiata* Ker. Gawl. we see a slight beginning of the foldings and the peltate basal flattening, and this species amongst the British Museum specimens most approaches *H. matopensis* in tunic form.

Owing to the separation of the base of the scale from the upper portion in these species, the tunics remain apparently one inside the other, the difference in length not being so marked, as only the upper portion persists as a ring.

In *Lapeyrousia Sandersonii* Baker, *abyssinica* Baker, *erythrantha* Klotsch, and *coerulea* Schinz, the scale-leaf is the same as in *H. matopensis*. In *Geissorhiza* the base of the scale-leaves is not peltate, but breaks off from the base as in some species of *Hesperantha*, and in one species as many as ten superposed tunics, graduated in size, were counted.

In considering the morphological value of the scale-leaves of the corm of *Hesperantha* in comparison with other members of the order, there is very little literature to fall back upon. Irmisch [1] found that the corms of *Crocus*, *Gladiolus*, and *Romulea* are the product of the preceding year's activity of the plant and belong to an axis of a next lower order. As these dry up the basal internodes of the flowering axis thicken into fresh corms, which give up their reserve storage material to a new generation (Spross-Generation) next year. This year's corm has usually three scale-leaves, which are inserted on the base of the corm, but buds arise in the axils of the scale-leaves. In an earlier paper Irmisch [2], in considering *Crocus*, finds no transition between the scale- and the foliage-leaves, a short internode separating the uppermost scale-leaf and the lowest foliage-leaf. Both forms of leaf die off later, but the bases of the former remain as a protective investment to the corm, which in a moist climate is thrown off every year. As the thickened portion of the stem in these cases consists of several internodes, no doubt the number of these latter determines the number of the scale-leaves. In *Hesperantha*, *Geissorhiza*, and *Lapeyrousia* therefore only one internode of the stem can be involved in the formation of the corm and we get one corresponding scale-leaf inserted on the base of the latter, the next season's corms arising in the axil of that leaf. In this case the scale-leaf does not rot away, but lignifies, remaining as a permanent investment or tunic.

In this peculiarity we find an interesting parallel amongst the grasses. Hackel [3], in a paper on some peculiarities shown by the grasses of dry climates, enumerates so-called 'Tunika-Gräser' as showing protective scale-leaf investment according to their habitat. The grasses of the fertile meadows of Northern Europe and North Asia are characterized by delicate scale-leaves which soon decay, and only the remains of one or two are to be found in the base of the culm. In grasses from dry climates, on the contrary, these scale-leaves thicken and persist, and, according to the type of persistent thickening, may be divided into 'Stroh-Tuniken' and 'Faser-Tuniken'. The former develop into hard, often smooth, entire straw-like scales, while in the latter the soft parenchyma breaks down, leaving the bundles isolated. This type may be carried further in the 'Fasernetz-Tuniken' in which the bundles anastomose again, forming horizontal as well as longitudinal threads, and both these types of tunic investment, persisting, result in many layers over the base of the culms, protecting the young shoots. These tunics also serve to collect water, as the superposed layers hold it tenaciously. Having had occasion recently to look through the Tropical and South African genera of the Liliaceae, Irideae, and Amaryllideae, it was very striking to see that what Hackel

[1] Irmisch, Morpholog. Beobachtungen (Berlin, 1855, pp. 10–25).

[2] Irmisch, Zur Morphologie der Knollen- u. Zwiebel-Gewächse (Berlin, 1850, pp. 89–94 and 166–70).

[3] Hackel, l. c.

describes so well for the grasses is exactly paralleled in the protective tunics of the bulbs, corms, or rhizomes of the representatives of those orders in those regions. The 'Faser-' and 'Fasernetz-Tuniken' are perhaps the most common, but in some genera, viz. *Geissorhiza* and *Hesperantha*, abnormally thickened 'Stroh-Tuniken' only are found. In the latter case the membranous unthickened parenchyma surrounding the strongly lignified internal portion of each superposed tunic must effectively increase the sponge-like efficiency of the whole water-holding structure. This would be a very important point for a plant like *H. matopensis*, which has to compete for water, at the most inauspicious time of the year, with perennial plants, having abnormally well-developed root systems already in possession of, and in intimate relation with, the soil particles.

JUSTICIA ELEGANTULA S. Moore.

This most interesting little plant is very widely spread in Southern Rhodesia and is recorded from Nyassaland as well (Nicholson, Herb. Kew). It was first collected by Dr. Rand in 1897 at Bulawayo and at Salisbury, and described by Mr. Moore[1] from those specimens, consisting of flowering shoots only. In 1902 it was collected again by Mr. Eyles in the Matoppo Hills, who sent a complete specimen to the British Museum; it did not, however, arrive until after the present investigation had been completed. In the present case[2] it was of very general occurrence in the Matoppo Hills, and a large quantity was noted on one of the sidings on the way up to the Victoria Falls, a fact which testifies to its wide distribution, though herbarium material is at present restricted to the specimens enumerated above.

The author first found it in August, growing in large patches on sandy veld, forming colonies suggestive of surface rooting origin. The little tufts of flowering shoots, scarcely a decimetre high, were dotted over these areas, where they were very conspicuous in the general deadness of surface vegetation, owing to their bright rosy pink flowers (Fig. 8).

Horizontally inclined, dead branches radiated from each tuft of flowering shoots (Text-fig. 8, *d.b.*). The latter, when taken up, were found to arise from winter resting buds of fleshy white radical leaves, forming sessile rosettes on a thickened rhizome beneath the surface (Text-fig. 8, *r.l.*). These rhizomes were not continuous, being limited to each group of shoots, and it was rather difficult to explain their origin. Finally, after some hunting, a vegetative shoot was found, with leaves still on it, which apparently explained matters, as it bore some roots at one of the nodes (Text-fig. 8, *n.r.*). On the flowering shoots the cauline leaves succeeding the hypogeal radical ones are at first small and linear, bearing

[1] Moore, Journal of Botany, XXXVIII, 1900, p. 204.

[2] Gibbs, l. c. p. 461.

the flowers in their axils (Text-fig. 8, *c.l.*). The radical leaves are minute, ovate-obcordate in shape (Text-fig. 9, *r.l.*). The first impression that they were water-storage organs was not borne out, as in section their tissues proved to be packed with starch. The structure is leaf-like (Pl. XII, Fig. 16), the palisade tissue being replaced by parenchyma in regular rows of cells on the dorsal surface (Pl. XII, Fig. 16, *p.*), while on the ventral surface typical mesophyll obtains, also packed with starch (Pl. XII, Fig. 16, *m.*).

FIG. 8. *Justicia elegantula.*

Stomata occur on the epidermis (Pl. XII, Fig. 16, *st.*), which is cuticularized, and some multicellular hairs are seen in the central depression (Pl. XII, Fig. 16, *h.*). It was impossible to get a good section of the cauline leaves, though the fact was established that the bilateral structure is maintained in them. From Text-fig. 8, *v.s.* 2, it will be seen that several buds remain dormant on the rhizome, and it is no doubt these which subsequently break away later on in the season, growing out into the vegetative shoots already referred to (Text-fig. 8, *v.s.* 1.). These shoots rooting at the nodes (Text-fig. 8, *n.r.*), the thick rhizome is elaborated previous to the development of the resting buds on its surface. The leaves on this vegetative shoot are very large, ovate, and about 3 cm. long. From the material in the British Museum, kindly placed at my disposal for drawing, later stages in the development of the flowering shoots could be followed. In specimens collected by Dr. Rand in September and October, the flowering shoots had elongated considerably, and the cauline leaves increased in size, whereas in December the erect growth was already lost and the shoot had dropped into a horizontal

FIG. 9. *Justicia elegantula*, Radical leaf.

position, the cauline leaves showing a very considerable increase in size, though still linear in outline (Text-fig. 10). The whole shoot at this stage assumes a dorsiventral habit, the little axillary flowering shoots rising erect from each node. It is the remains of these decumbent flowering shoots which form the radiating dead branches in Text-fig. 8, *d.b.*, in which no trace of nodal rooting was observed either in the field or in the material brought back. The cauline leaves also in this case, though they increase so considerably in size, remain more linear than the ovate leaves on the purely vegetative shoot, on which there are no flowering axes to exhaust vitality. The later development of vigorous vegetative shoots brings this highly specialized *Justicia* into line with the general characteristic type of vegetative activity already referred to, by which plants requiring a long period of development economize their resources by taking the process in two stages. This habit is possibly stimulated in herbaceous plants by the annual veld fires, as it ensures a double chance in case of premature destruction of the purely reproductive shoots. That it forms the direct response to the prevailing climatic and edaphic conditions is shown by the wide adoption of the principle, in one form or another emphasized in the very early flowering of nearly all the trees, many shrubs, and those herbaceous plants and bulbs the organization of which calls for a large area of assimilating surface to elaborate next year's supplies. The decumbent shoots also form a very general adaptation, in this case peculiarly appropriate, for retarding evaporation, and ensuring a shade area at the stage when the winter buds are being laid down on the rhizome.

In its peculiar complexity of organization *Justicia elegantula* not only occupies an isolated position in its own genus, but

FIG. 10. *Justicia elegantula.*

also within the whole alliance. In the Acanthaceae tuberous development is very rare, and Lindau[1] only refers to *Ruellia tuberosa* as an example, in which case it takes the form of local swelling of the roots. The peculiar development of specialized leaves for nutritive purposes finds no parallel in this order, though rooting at the nodes is a general feature. The production of resting buds is well known in certain families, notably species of *Epilobium*, i. e. *E. parvifolium* Shreb., *E. montanum* Linn., and *E. lanceolatum* Sebast. et Maur, where the autumnal stolons produce fleshy white rosulate leaves, very similar in appearance to those occurring in the present case. Goebel[2] quotes *Androsace sarmentosa* Wall. as forming resting leaf rosettes consisting of leaves very different from the foliage-leaves. In *Utricularia* and *Myriophyllum* winter buds or turions are formed, but these examples serve also for vegetative reproduction, as they may be detached from the parent plant, which is not the case with sessile leaf rosettes, which are organs modified as reserved storage-tissue for the purpose of accelerating next year's growth.

In conclusion we may point out that in two plants of most widely differing organization and systematic position, exposed to the same physiological but rather divergent edaphic conditions, the direct response is on the same lines in both cases, and in the same direction, viz. to the general or physiological stimulus rather than to individual requirements. The important organogenic modifications involved are attained in both cases by the specialization of some organ or organs as a reserve food store, thus providing not for present requirement, but for future need. To ensure next season's rapid growth on the most economical lines, present opportunities are utilized to the utmost, but the necessity of elaborating contrivances to meet potential adverse circumstances represented by a prolonged physiological drought is discounted, and the plant secures itself against the periodic recurrence of dry seasons by the cessation of all vegetative activity in the annual dying down of its aerial shoots. It is the mean of physiological environment and not the extreme which here determines biological modification. In the case of *Fuirena Oedipus*, which was in flower in September, the favourable growing period would probably cease in January, when the summer rains begin to affect the volume of water in the river (p. 191). From then till June it would be exposed to a continual downpour, with its rhizome and roots in standing water. For *Justicia elegantula* the conditions are apparently different, though physiologically the same, as it is one of the few surface-rooting veld plants. Amongst these latter the absence of stolons or surface-rooting runners was very conspicuous, though a widely spreading system of dorsiventral shoots was such a very general adaptation. *Listia heterophylla* E. Meyer, on moist sand-banks near streams, roots very freely at the nodes, but the same plant on the veld shows a very

[1] Lindau, Nat. Pflanzenfamilien, iv. 3 b. [2] Goebel, Organography of Plants, p. 398.

limited branching system and, as far as early spring conditions go, no tendency to nodal rooting. *Lobelia thermalis* Thunb., a plant of very wide South African distribution, but in this case only found growing over rocky banks by a stream, was a very free surface-rooter, sending the rootlets down between the fissures of the rocks. On the veld the absence of surface moisture is no doubt a deterrent to this habit, and *Justicia elegantula* has had to adapt itself to surface-rooting by so organizing its vegetative growth that the energies of the plant can be directed towards the elaboration of a tuberous rhizome, and on this the starch-packed radical leaves are laid down. The plant is therefore doubly ensured against the long drought and the accompanying absence of moisture in the sandy surface of the soil. That the results are successful, is well shown in the wide distribution of the plant already referred to (p. 201), and it may be put down as one of the commonest Rhodesian types.

Hesperantha matopensis calls for no further remark. The development of its corm-tunics is on lines common to tropical and South African bulbous plants, which, as Hackel has shown in the case of grasses, is characteristic of dry climates in general, and is correlated with the supreme necessity of ensuring a sufficient store of moisture round the roots and young shoots.

Finally, my thanks are due to Dr. Rendle, of the British Museum, for kindly placing material at my disposal, and to Professor Farmer for his advice and criticism in the course of this work.

ROYAL COLLEGE OF SCIENCE.
May 2, 1907.

EXPLANATION OF PLATES XI AND XII.

Illustrating Miss L. S. Gibbs' Paper on new Rhodesian Plants.

O. ovule. *r.* raphe. *v.b.* vascular bundle. *d.s.* dead shoots. *b i.* basal internode. *sch.* sclerotic tissue. *e.* endodermis. *p.* plexus of bundles. *a.v.b.* amphivasal vascular bundle. *p.x.l.* protoxylem lacuna. *st.* starch. *t.s.* tannin sac. *l.t.b.* leaf-trace bundle. *c.b.* cortical bundle. *m.b.* medullary bundle. *b.h.* base of hair. *v.e.* ventral epidermis. *d.e.* dorsal epidermis. *a.c.* air canals *l.p.* lignified parenchyma. *w.s.* water-sheath. *m.* mesophyll. *p.* palisade. *st.* stomata.

PLATE XI.

Fig. 1. A portion of a plant of *Fuirena Oedipus* showing the rhizome bearing old and young flowering and vegetative aerial stems, the basal internodes of which are swollen, forming starch storage-tissue; the old stems to the right show reduction in size owing to the resorption of the reserve material. Reduced.

Fig. 2. A flowering shoot detached from the rhizome.

Fig. 3. A flowering spikelet detached from the inflorescence. × 16.

Fig. 4. A flower in the axil of a bract. Mag.

Fig. 5. A fruiting bract. Mag.

Fig. 6. A flower showing the three perianth segments alternating with the three stamens, and the triangular ovary. × 75.

Fig. 7. A longitudinal section through the ovary, showing the erect anatropous ovule. × 75.

PLATE XII.

Fig. 8. Part of a transverse section of the rhizome of *Fuirena Oedipus*, showing the broad cortex with hypodermal sclerotic ribs, the endodermis reinforced by a zone of sclerotic cells, and the solid central cylinder with amphivasal fibro-vascular strands, more numerous towards the periphery. × 110.

Fig. 9. Part of a transverse section through the swollen basal internode, showing the proliferation of cortical tissue for starch storage purposes, a peripheral ring of leaf-trace fibro-vascular bundles, an inner cortical ring, and the medullary bundles, which are connected by thin plates of parenchyma across the large air-canals of schizogenous origin of which the medulla is composed. × 65.

Fig. 10. A radial longitudinal section through the basal internode, showing the insertion of the leaf-trace bundles on to the cortical bundles, the limits of the starch storage cortical tissue, and the medulla with its large air-spaces. × 16.

Fig. 11. Half of the transverse section of the stem taken through the third internode from the base, showing the hypodermal ribs, cortical parenchyma reduced to two or three layers, which show starch contents where they pass over the cortical bundles, the latter alternating with lysigenous air-spaces, and a medulla identical in structure with that of the basal internode. × 110.

Fig. 12. Part of a transverse section of the leaf, showing the differentiation of the epidermal cells on the dorsal and ventral surfaces, the connexion of the water-sheath surrounding the vascular bundles of the epidermis, and the lysigenous air-spaces which replace the mesophyll tissue of the leaf. × 1000.

Fig. 13. Transverse section of half of the leaf, showing the stomata limited to the dorsal surface and the very large water-cells and unicellular trichomes on the ventral surface. × 75.

Fig. 14. Part of a longitudinal section of the leaf, through an air-canal, showing the breaking down of the mesophyll tissue and the papillose outgrowth of the cuticle of the epidermis. × 1000.

Fig. 14 A. Surface view of stoma.

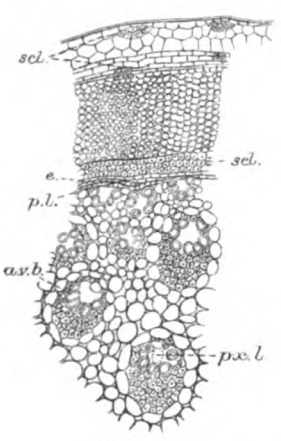
Fig. 15. Part of a transverse section through a developing leaf of *Carex stellulata* in the very youngest stage, showing the mesophyll tissue not yet broken down. × 1000.

Fig. 15 A. Part of a longitudinal section of a very young leaf of *Carex stellulata*, showing the mesophyll tissue beginning to break down. × 1000.

Fig. 16. Part of a transverse section through a fleshy radical leaf of *Justicia elegantula*, showing starch storage in its tissues. × 1000.

Fig. 17. A transverse section through one of the laciniate teeth of a scale-leaf of *Hesperantha matopensis*, showing the lignified inner layers of parenchyma.

GIBBS — RHODESIAN PLANTS.



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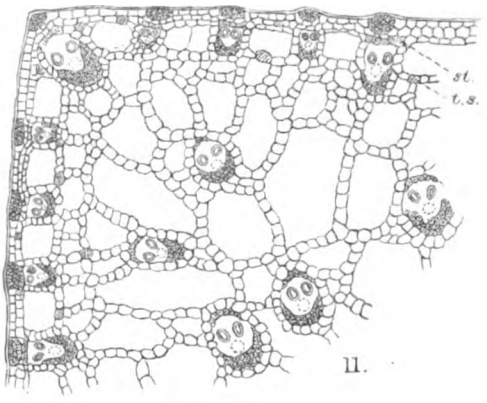

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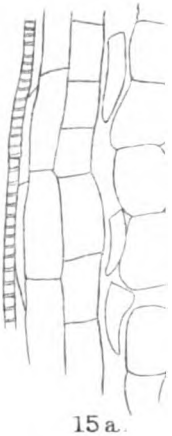

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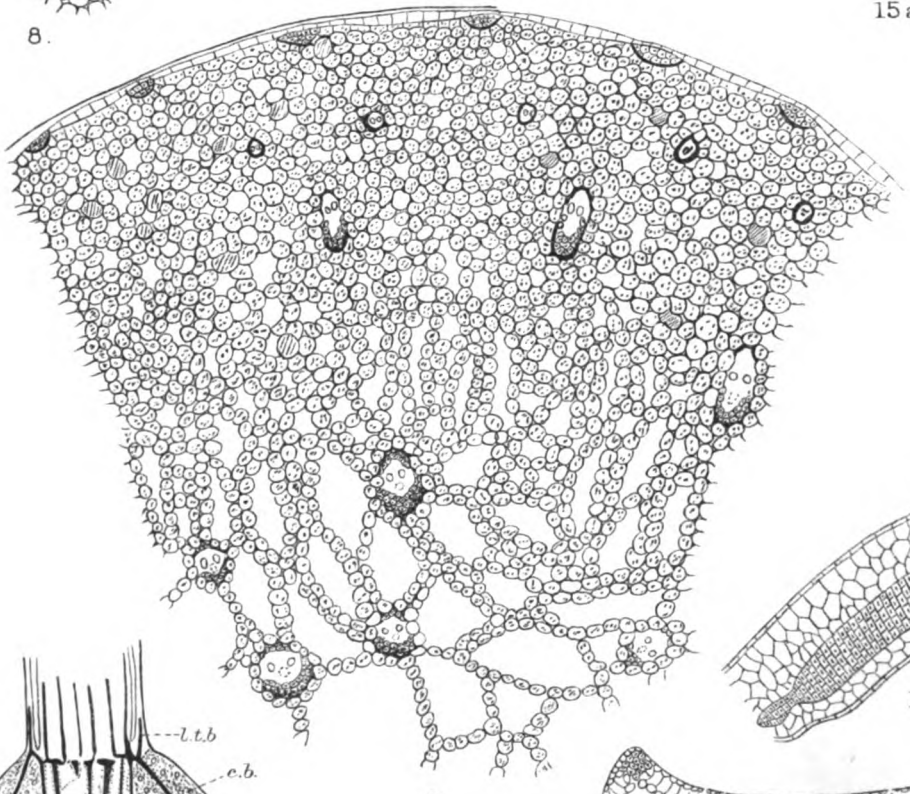

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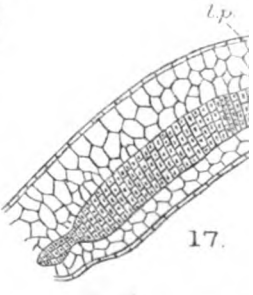

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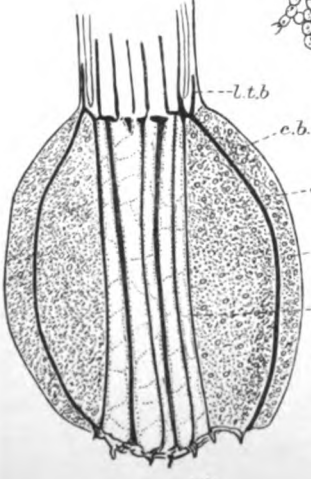

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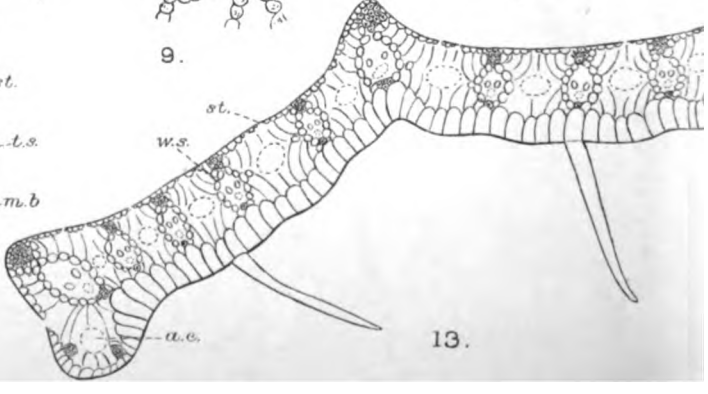

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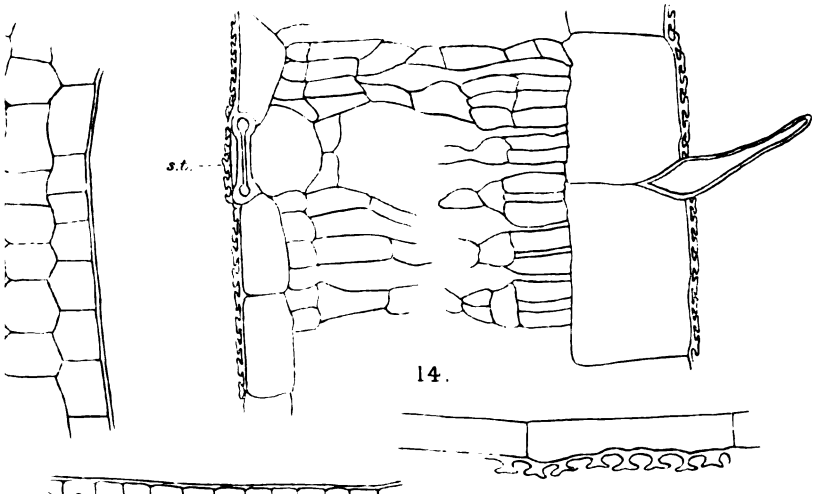
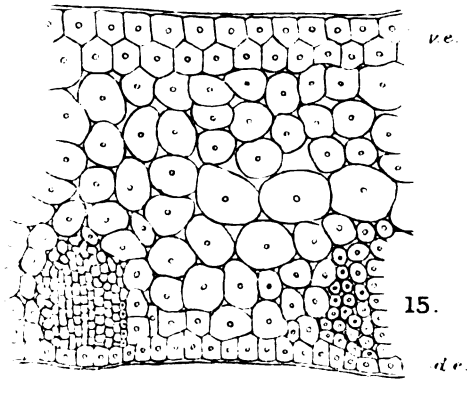


14.

14 b.

14 a.



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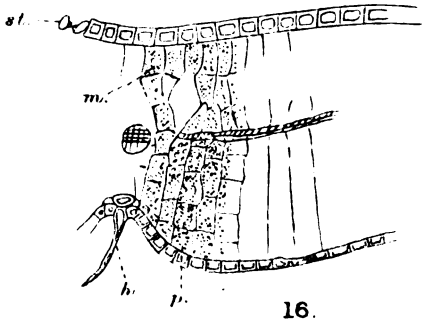

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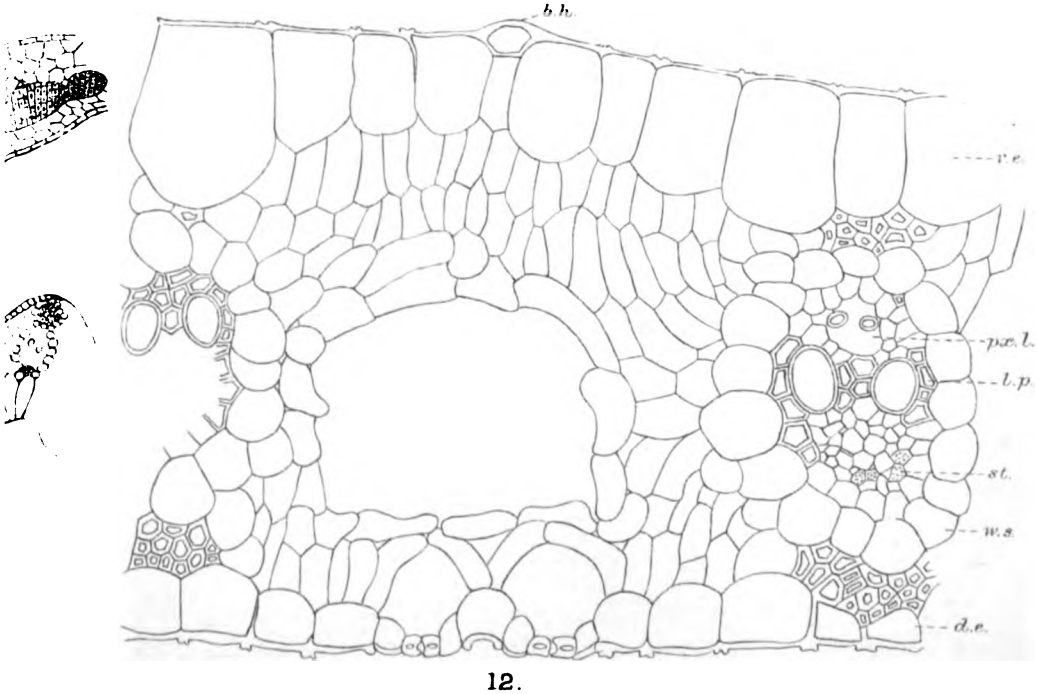


12.

On the Structure of the Leaf in Cretaceous Pines.[1]

BY

EDWARD C. JEFFREY

Professor of Plant Morphology in Harvard University.

With Plates XIII and XIV.

THE leaf in the Gymnosperms has been the subject of many investigations, and it is now recognized that the fibrovascular structures of the foliar organs in this group are phylogenetically important. It is the purpose of the present communication to call attention to certain structural features of the leaves in Cretaceous Pines and allied forms, which appear to have an important bearing on the much disputed problem of the origin of the Coniferales.

It will be appropriate to commence this record with the description of those pine-like leaf remains which appear to present the most primitive characters. Fig. 1, Pl. XIII, shows a magnified external view of a short-shoot or brachyblast of an Abietineous species, which is rather rare in the Kreischerville deposits, from which all the material described in the present connexion has been derived. It will be noticed at once that it differs so strikingly from the fascicular shoots of any living species of *Pinus*, that, judging from external features alone, it might well be doubted whether our specimen was a short-shoot at all. The specimen represented in Fig. 1, Pl. XIII, shows at the base scars of fallen leaves which in this case are not foliage leaves, but are of the nature of bracts. Above the denuded region is a number of bracts still *in situ*. At the very top of the figure the brachyblast presents a truncated appearance and is crowned by the broken bases of a number of true foliage leaves. Fig. 3, Pl. XIII, shows another short-shoot of this species, which is in a much better condition

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 10.

[Annals of Botany, Vol. XXII. No. LXXXVI. April, 1908.]

of preservation. Much less of the base of the brachyblast is retained, and the sheathing bracts as a result present only a short segment. Above them, however, the foliage leaves are represented by much more elongated bases than in the last-mentioned figure. It may be made out that the fascicular leaves are more numerous than they are in any living Pine. Further, they are not in definite number as is the case in existing species of *Pinus*. The sheath in our specimen is composed of the same loose scales as are found in the sections *Strobus* and *Cembra* among living Pines; but its component bracts, like the fascicular leaves, are much more numerous than they are in any living species of *Pinus*. Moreover they are not deciduous, as is true of the elements of the fascicular sheath in the sections *Strobus*, *Cembra*, &c.

Fig. 2, Pl. XIII, represents a transverse section through the bases of the fascicular leaves in another specimen. The section does not include all of the leaves of the fascicle, since some of these have been removed in process of fossilization. The second projection on the lower left side of the figure represents the growing point of the short-shoot, which, contrary to the conditions found normally in living species of *Pinus*, clearly persists, and in some of our specimens is protected by a covering of thin scale-like bracts. Regarding the remains of the growing-point of the specimen shown in cross-section in the figure as its organic centre, it is clear that there must have been more than twenty-five leaves attached to the uninjured brachyblast. The number appears, however, to vary considerably, so far as we are able to judge from the rather fragmentary specimens at our disposal. As a result of the presence of a large number of leaves in the fascicles, these present, in the case of the internal leaves, a very different contour from those of the normal short-shoots of any living species of *Pinus*; for instead of being bounded by either one or two plane surfaces and one curved one, as is invariably the case in the fasciculate leaves of existing Pines, the internal leaves in these polyphyllous brachyblasts are polygonal in outline and bounded on all sides by plane surfaces. Another feature of contrast to living Pines is presented by their phyllotaxy, for their fasciculate leaves are arranged spirally and not verticillately on the short-shoots.

Before discussing further the internal structure of these interesting short-shoots, it will be well to refer briefly to *impressions* of a somewhat similar appearance described by other authors. Fontaine, in his monograph on the Potomac Flora, figures certain remains which he refers to Heer's Jurassic genus *Leptostrobus* ('The Potomac or Younger Mesozoic Flora', Monographs of U.S. Geological Survey, XV). These he describes as presenting both fasciculate leaves and others, arranged spirally at intervals on the relatively main branches, the latter comparable to the primary leaves found in the seedling, and occasionally as the result of injury in the adult, of living Pines. The leaf-fascicles in Fontaine's species are characterized,

as in our specimens, by the large number of fascicular leaves. In the Kreischerville deposits only short-shoots have been found as yet. Our specimens also strongly resemble *Pinites Solmsi* of Seward (British Museum Reports, Fossil Plants of the Wealden, Part 2). His Fig. 2, Pl. XVIII, shows leafy short-shoots, which must have presented a striking resemblance to our specimens in the living state, since they seem to have had the same long bractigerous base and numerous fascicular leaves.

Turning our attention now to the internal structure of the leaves and axes of these curious brachyblasts, we find in Fig. 4, Pl. XIII, a magnified view of one of the outer leaves shown in Fig. 2. The figure represents the leaf in its morphologically correct position with the xylem uppermost. The wood is only moderately well preserved, and the phloem has entirely disappeared, as is generally the case in Abietineous leaves from the Kreischerville deposits. The remainder of the leaf is composed of sclerified tissues, which contain towards the slightly flattened margins of the leaf two resin-canals. These present a remarkable contrast to those found in the fascicular leaves of living Pines, in the fact that they penetrate to the very base of the leaf and are occluded in this region by tyloses. The latter can be clearly distinguished in the figure. It has not been possible to follow with certainty the foliar resin-canals into the cortex of the short-shoot itself on account of the scantiness of well-preserved material; but it is highly probable that, unlike the fascicular leaves of living Pines, those of *Prepinus statenensis*, as we propose to call our specimens, had their resin-canals continuous with those of their axis and thus resembled the primary leaves of living Pines. The leaf-trace in *Prepinus statenensis* remained undivided in its course through the cortex of the axis of the short-shoot, and in this respect resembled the fascicular leaves of the living species of *Pinus* belonging to the sections *Strobus*, *Cembra* and *Crayopitys*.

An important feature of the leaf-trace in our species is the fact that there is a considerable amount of true centripetal wood present. In Fig. 4, Pl. XIII, this can be distinguished by attentive examination, on the right of the fibrovascular bundle. Fig. 5 shows the bundle of this leaf more highly magnified. The preservation is unfortunately not very good; but nevertheless, on the right hand, radiating masses of xylem tissue, passing away centripetally from the face of the considerable mass of centrifugal wood, can be made out. These masses of centripetal wood, starting with a narrow base, expand in a fan-like fashion towards the upper surface of the leaf. The presence of this well-developed centripetal xylem in the leaf of a Cretaceous Pine-like Conifer appears to be not without significance, since, so far as is known to the writer, it is the first recorded case of the presence of such tissues in a Coniferous leaf, living or extinct. The transfusion tissues are intentionally disregarded in making this statement, since, as will subsequently be shown, these cannot properly be considered as representing

the true centripetal wood, as has been maintained by Worsdell, and more recently by Bernard and others.

Before turning our attention to the structure of the higher portion of the leaf in the species under consideration, it will be well to examine briefly the organization of the axis of the short-shoot. Fig. 8, Pl. XIII, shows the manner in which the large traces of the fascicular leaves pass from the central cylinder of the brachyblast. It is to be noted that they do not divide into two, as is the case in the primary leaves of the seedling even of the *Strobus* group of Pines. This is additional evidence that we have really to do with a short-shoot and its appurtenant leaves. Fig. 6 shows the structure of a section through the woody cylinder of the short-shoot of the same specimen which furnished Figs. 2, 4, and 5. The magnification is not sufficient to show the details of structure; but it is possible to make out that there are islands of sclerenchyma, appearing as dark spots in the pith. This feature is not found in living representatives of the *Strobus* and allied sections of living Pines; but is common among the Hard Pines with three and five fascicular leaves. Fig. 7 shows a portion of the woody cylinder of the short-shoot more highly magnified. Although the enlargement is considerable the tracheids appear small. The most important feature of this figure is the presence of a resin-canal in the upper portion stopped with tyloses. The presence of this phenomenon in the wood of a shoot showing but a single annual ring makes it certain that we can have to do with nothing but the axis of a deciduous shoot or brachyblast. Thus the histological structure of the axis of these curious leafy shoots, which we have named *Prepinus statenensis*, makes it clear that they are brachyblasts of a primitive and somewhat generalized type of Cretaceous Pine-like Conifer.

In view of the general tendency to regard the Araucarians as the most ancient Conifers, the radial pitting of the tracheids of the brachyblasts in this species is of special interest. Fig. 9, Pl. XIII, represents the ends of several tracheids of *Prepinus statenensis* as seen under a magnification of 180. It is to be expected, if the Araucarineae really represent the most primitive type of Conifer, that some evidences of Araucarian structure should be found in the wood of this ancient Pine-like Conifer. The crowded pitting in the ends of the tracheids is purposely chosen for our Fig. 9, since one might expect to find the flattened or angular Araucarian type of pit exemplified under these conditions if at all. It will be noticed, by examining the figure, that the pits do not become more than slightly oval as a result of extreme approximation and in no case show the angularity or flattening of the Araucarian type. Fig. 10 shows the pits very highly magnified, to enforce the truth of this statement. On the left can be seen indications of the presence in the walls of the tracheids of the folds of Sanio between the adjacent pits, especially in the lower part of one

of the tracheids. This is the first occasion on which the author has observed this phenomenon, so common in living Conifers other than the Araucarineae, in a Mesozoic lignite. The entire absence of all indications of a transition towards the Araucarian condition of pitting in the oldest structurally known Abietineous Conifer furnishes a strong argument against the Abietineae having come from an Araucarian stock. On the other hand, in the wood of numerous species of the older Araucarians, which have been investigated for the first time structurally, in the case of leafy branches, by the author, there is a very marked departure from the Araucarian type of flattened or angular pits, towards the rounded bordered pits present in the remaining Coniferales, notably in the Abietineae. This transition towards the Abietineous condition in the radial pitting of the tracheids is paralleled by marked Abietineous characters in the wound reactions, and in some cases by the ray structure found in the case of the older Araucarians of the Cretaceous. It may accordingly be stated, in passing, that there is much better anatomical evidence from the Cretaceous for the Araucarineae having come from the Abietineae than for the opposite view, which is implied by the hypothesis that the Araucarians are the most ancient of living Conifers. Further consideration of this subject may advantageously be deferred until the end of the article.

Fig. 13, Pl. XIII, is a magnified representation of a transverse section of a leaf resembling that of *Pinus*, which is considered, on grounds to be stated below, to belong to *Prepinus statenensis*. The contour of the leaf is very different from that found in any living Pine, for instead of having one curved face and one or two plane surfaces, as is the case in the fascicular leaves of *Pinus*, we find five approximately plane surfaces. Any departure from the planar condition is probably to be explained as the result of the vicissitudes accompanying fossilization. On the margins of the angular leaf are to be seen two resin-canals. The fibrovascular bundle is single, as in the sections *Strobus*, *Cembra* and *Caryopitys*, of living Pines, and entirely lacks the phloem, which has disappeared through decay. The bundle is bounded by a dark zone which completely surrounds it, the like of which has not been found in the fascicular leaf of any living Pine. Outside the dark sheath just mentioned is a thick zone of transfusion tissue, made up of coarsely pitted transfusion cells, without any admixture of parenchyma, such as is found abundantly in the tissues surrounding the leaf-bundle in living Pines. This entire exclusion of parenchymatous elements from among the short tracheid-like cells, which constitute the transfusion tissue, has an interesting parallel in the complete absence of parenchyma (exclusive of that found in the rays) in the secondary wood of the older Gymnosperms, and is probably in both cases to be regarded as a primitive feature. Another remarkable contrast with living Pines offered by our species is the absence of any indication of an endodermal sheath separating the bundle and its associated

transfusion tissues from the mesophyll. Still another noteworthy feature is the presence of strong hypodermal ribs beneath the epidermis. Fig. 11 shows another section of the same leaf still more highly magnified. All the features signalized above can now more clearly be made out. The transfusion tissue is particularly obvious in this figure. Fig. 14 shows the fibrovascular bundle somewhat highly magnified. To the left is a cavity, which marks the position of the phloem, now entirely gone. The dense although not very thick sheath, which surrounds the bundle proper, can be clearly distinguished in this figure. The most interesting feature of the figure, however, is the structure presented by the wood. It can be made out that there is a *large amount of centripetal xylem present.* It will be observed that the centripetal elements, in the centre of the bundle, begin about the middle of a radius drawn through the xylem and pass with a gradual enlargement of their lumen towards the dark sheath already described, upon which as a rule they immediately abut. Fig. 15 shows the central portion of Fig. 14, much more highly magnified. The seriation of the elements is particularly clear in this figure, as well as their relation to the peculiar bundle sheath. The centrifugal xylem is not broken into separate strands as is the centripetal wood. It does not come into direct relation with the thick-walled sheath above mentioned, except on its flanks. In other regions of the xylem the relation between the centrifugal elements and the transfusion tissues is brought about through the centripetal xylem. Fig. 12 shows a longitudinal section through the leaf-bundle somewhat to one side of the centre. The tissues do not appear as well preserved in this plane of section as in the transverse ; but it can nevertheless be made out that typical mesarch structure is present. The ringed and spiral protoxylem elements occupy the centre of the figure, and on the left pass into reticulate elements, which are at once succeeded by pitted tracheids of the centrifugal wood. On the opposite side of the protoxylem are the thinner-walled and less well preserved elements of the centripetal wood, which are entirely pitted tracheids. On the extreme right is to be seen a portion of the very dense bundle sheath. This sheath is so darkly coloured in the fossil that it is almost impossible to resolve its structure even after the prolonged action of such a bleaching agent as chlorine water. It is possible, however, to make out with sufficient pains that it is composed of very thick-walled transfusion elements, with small bordered pits. The elements of the dense transfusion sheath are very much longer and of much narrower lumen than are the more external transfusion cells. They in fact present a certain resemblance to tracheids. The broad zone of transfusion cells surrounding the dense sheath just described is made of cells of much thinner walls and only slightly elongated longitudinally. As has been mentioned at an earlier stage, these are all pitted with bordered pits, which are about twice as broad as those found in the cells of the transfusion

sheath. There is no indication of an endodermis separating the transfusion tissues from the mesophyll as in modern Pines. There is possibly a correlation between the presence of the dense sheath surrounding the bundle and the absence of the endodermis. It is not improbable that the inner sheath, with its thick walls and small bordered pits, may have exercised a beneficial restraining influence in the case of excessive transpiration. Whatever the physiological function of the inner transfusion sheath may have been, we have to go for a parallel to it, significantly enough, to the Cordaites, as will be noted in a subsequent paragraph.

Before proceeding to the discussion of the resemblance of the leaf-bundle in the species which has just been described, to the foliar strands in Cordaites, it will be well to establish the relation of the isolated leaves to the short-shoots which have been considered in earlier paragraphs. From the description of the attached fasciculate leaves of *Prepinus statenensis* given above, it appears that the isolated leaves under discussion agree in the following features: the possession of centripetal wood; the single foliar bundle; the paired resin-canals; and finally, a very important feature, the polygonal outline of the leaf-section, resembling that found in the internal leaves of the brachyblasts of *Prepinus statenensis*. The only very noticeable difference is the presence of transfusion tissue in the case of the isolated leaves, which is absent in the attached bases of the leaves of the short-shoots. This feature is, however, of slight significance, since it is paralleled in the case of *Pinus*, where the transfusion tissue does not appear in the lowermost part of the fascicular leaves. It seems reasonable to conclude from the features of close agreement described above, and especially as *Prepinus statenensis* is unique among the remains of Abietineous Conifers found in the Kreischerville deposits, that the isolated leaves and the short-shoots referred to *Prepinus statenensis* belong together.

A few years ago Miss Stopes described an interesting peculiarity of Cordaitean leaves referred by her to the *Cordaites principalis* of Renault. In Cordaitean leaves, as is well known, the centripetal wood becomes continuous with a zone of transfusionary elements which completely surround the bundle, including the phloem as well as the xylem. Between the protoxylem and the phloem and on the dorsal side of the bundle, there is sometimes present more or less centrifugal wood, which is, however, always less abundant than the centripetal xylem. Miss Stopes (New Phytologist, vol. ii, pp. 91–8, Pl. IX) has shown that in *Cordaites principalis*, what had been taken by Renault for centrifugal wood, was in reality a sheath of elongated transfusion elements, *surrounding the phloem on its dorsal surface* and not interposed *between* the phloem and the centripetal wood, as should be the case were it really centrifugal wood. This sheath she regards as equivalent to the 'primitive transfusion tissue' of Worsdell, the larger-lumined, less prosenchymatous tracheidal sheath external to it being the

counterpart of Worsdell's 'peridesmic transfusion tissue'. Through the kindness of Professor Oliver I have had the opportunity of examining the beautifully preserved material upon which Miss Stopes's observations were made. Fig. 16, Pl. XIV, shows a leaf-bundle from this material somewhat highly magnified. The fibrovascular strand is surrounded by a cordon of large, apparently empty cells, which constitute the outer or 'peridesmic' transfusion tissue. The centripetal xylem lies on the upper side of the bundle, within the 'peridesmic' sheath just described. The bundle illustrated in Fig. 16 presents in essential features a striking resemblance to Fig. 14, the most marked differences being the presence of centrifugal xylem in *Prepinus*, and the fact that the inner sheath of the transfusion tissue does not extend over the posterior region of the xylem in *Cordaites*. In the sections loaned by Professor Oliver there was a good deal of variation in the extent of the inclusion of the centripetal xylem by the inner thick-walled transfusional sheath, in some instances there was only a single tracheid between the mass of centripetal xylem and the large-lumined outer sheath of transfusion tissue. In any case there is a very striking resemblance between the foliar bundles in *Prepinus* and *Cordaites*. This resemblance is none the less significant because of the opinion frequently expressed in recent years by competent investigators, that the Coniferales are descended from the *Cordaites* or from a Cordaitean plexus.

In Fig. 17, Pl. XIV, is shown a section through the basal region of a four-leaved fascicle of a contemporary Cretaceous Hard Pine. The needles are surrounded by the close membranous sheath of the Hard Pines and have the double leaf-trace which is characteristic of that group. By examination it will be seen that the leaves are without resin-canals in the basal region shown in the figure, and in this respect conform to the fascicular leaves of living Pines and differ from *Prepinus statenensis* described above. The double bundles of the leaf-traces are surrounded on the outer side in the region of the vanished phloem as well as in the region of the xylem by a sheath, comparable to that found in *Prepinus statenensis*. This sheath sends off a tongue which is inserted between the two bundles, forming a complete septum of separation. Fig. 18, Pl. XIV, shows the structure of a detached leaf from a Cretaceous Pine with a two-leaved fascicle. It is highly probable, from the presence of double bundle in the leaf in question, that it represents a Hard Pine. In Fig. 19, Pl. XIV, appears in transverse section the leaf of another species of bifoliar Pine which is characterized by the presence of a single fibrovascular bundle and consequently in all probability belongs to the general group of Soft Pines. In both these figures it is to be noted that the transfusion tissue is of the same type as in *Prepinus statenensis*, being composed of an inner dense sheath and a broad zone of outer large lumined transfusion elements.

In the case of Figs. 18 and 19 it is, however, by no means certain that there is no parenchyma intermingled with the outer transfusion elements, although the tracheidal cells are in any case infinitely more abundant than they are in living Pines. In the two species under discussion the endodermis is not clearly marked and may well be absent. The mesophyll is very scanty in amount, and lacks the infolded walls which characterize it in living species of *Pinus*.

Fig. 20, Pl. XIV, shows a somewhat smaller leaf of a Cretaceous Pine than those shown in the two previous figures, but its organization is of the same general type. The state of preservation is more favourable to the differentiation of the inner transfusion sheath than in the foregoing species. It may also be somewhat clearly distinguished, although the magnification is low, that the xylem of the bundle is entirely centrifugal, a condition which we have found to be present in all true Pines from the Middle Cretaceous of Staten Island. Fig. 21, Pl. XIV, shows a highly magnified longitudinal section of the bundle in this species. The inner transfusional sheath appears as very thick-walled, dark-coloured cells, which in the vicinity of the protoxylem are very considerably elongated and possess a very narrow lumen. Passing towards the left in the figure, the transfusion elements become at first more nearly isodiametric and then much thinner-walled. On the extreme left they have suffered disorganization. On the side of the xylem near the inner transfusional sheath are to be made out the somewhat disorganized ringed and spiral elements of the protoxylem. There is no indication whatever of the presence of true centripetal elements, such as are to be found in the foliar bundles of *Prepinus* as described above. Further to the right the centrifugal elements become reticulated and finally pitted, although the latter condition is not clearly shown in the figure.

Fig. 23, Pl. XIV, shows the cross-section of another leaf of *Pinus* belonging to still another Cretaceous species, which, we may judge from the angle made by its two plane surfaces, came from a five-leaved fascicle. In this case the inner sheath of the transfusion tissue has become almost obsolete and appears most clearly on the side of the vanished phloem and between the bundles. Fig. 22 reproduces the transverse section of another leaf, which probably belonged to a three-leaved fascicle. The inner transfusional sheath in this case can be distinguished rather better on the side of the phloem than in the last described species. It has suffered disintegration between the bundles, but again appears at the back of the protoxylem. Fig. 24 shows the transverse view of a very large leaf which has become considerably flattened in process of fossilization. The inner transfusional sheath can be clearly distinguished except on the flanks of the bundles, where in all species it tends to be thin.

It is obvious from the data supplied in the foregoing paragraphs that the leaves of species of true Pines from the Middle Cretaceous of Staten

Island differed from modern Pines, (1) in the better development of the transfusion elements around the bundle; (2) in the differentiation of the transfusion elements into an inner sheath composed of elongated tracheidal elements and an outer, much broader zone made up of more nearly isodiametric elements with thinner walls; (3) in the probable absence of an endodermis; and (4) in the absence of infolding of the walls of the mesophyll. They resembled their modern descendants in possessing a fixed small number of verticillate fascicular leaves for the brachyblasts of each species. The growing-point of the brachyblast in the true Pines of the Cretaceous often persisted, although the author in no case has found it to be as prominent as in *Prepinus*. The wood of the leaf-bundles was entirely endarch. *Prepinus statenensis* differed from other Cretaceous Pine-like Conifers in the possession of true centripetal wood, such as has been described in the leaves of no other Conifer living or extinct. The fascicular leaves were attached in an indefinite large number spirally to the brachyblast, which was deciduous as in the true Pines, but differed from them in having a prominent persistent growing-point covered with protective scales. The transfusion tissue of *Prepinus* has the same general organization as in Cretaceous species of *Pinus*, but it was much more abundant, and the outer zone had no admixture of parenchymatous elements.

Conclusions.

The presence of centripetal wood in *Prepinus* in the present state of our knowledge can scarcely be interpreted in any other way than as the persistence of an ancestral character possessed by the parent stock of the Abietineae. If it be conceded that the centripetal wood which is found in the leaves of *Prepinus* is a vestigial feature, it cannot well be denied that the double transfusionary sheath accompanying it in that genus and still retained in the Middle Cretaceous representatives of the true Pines is susceptible of a similar interpretation. This view of the matter is rendered still more probable because of the close agreement of the presumably primitive Abietineous leaf-bundle with that found in certain Cordaites which are regarded, by those whose studies fit them to judge, as the ancestral stock from which the Coniferales have been derived. If we may venture to depict the phylogenetic development of the Coniferous foliar bundle in the light of the new facts supplied by the study of their Mesozoic representatives, it would appear to be somewhat as follows. The ancestral Conifers, closely allied to the Cordaitales, possessed foliar bundles characterized by the presence of centripetal xylem and transfusion tissue of a complex type, consisting of a double cordon, an inner sheath composed of elongated tracheidal elements, and an outer jacket of ordinary transfusion cells without any admixture of parenchyma. In the course of subsequent

evolution the centripetal wood was the first archaic feature to become blotted out. Its disappearance was complete and occurred at a comparatively early stage of Coniferous history. At a later stage the inner transfusion sheath followed the centripetal wood into oblivion, although even in modern Conifers traces of it may still be found in the region of the protoxylem. As the result of the disappearance of the centripetal wood and the inner transfusional sheath, the centrifugal wood became ultimately directly continuous with the jacket of ordinary transfusion tissue, the outer transfusion sheath of the more ancient Abietineae. The outer or 'peridesmic' transfusion zone in the course of these changes became more and more degenerate and intermingled with parenchyma cells, and came to possess the structure shown in the vegetative leaves of modern Pines. The outer sheath is also persistent to some extent in the leaves of the Araucarineae, but even the older known representatives of this family show no indication of the existence of true centripetal wood or of an inner transfusion sheath.

The new data supplied by present investigation and by other recent researches on living and extinct Coniferales seem to strengthen the hypothesis put forward over two years ago by the author, to the effect that the Abietineae are a very old, if not the oldest, family of the Coniferales. It specially stands out in connexion with these results, that the Abietineae must be considered more primitive than the Araucarineae, which are generally regarded as the ancestral Conifers. The arguments for this view may be summarized as follows:—

1. The possession on the part of the Abietineae of marked vestiges of a double leaf-trace, such as is almost universally characteristic of the older Gymnosperms.

2. The presence of true centripetal wood in the genus *Prepinus*, which may be regarded with a strong degree of probability as the ancestor of the living genus *Pinus*.

3. The marked and detailed resemblance of the foliar bundle in *Prepinus*, not only in the presence of centripetal wood, but also in the complex double sheath of transfusion tissue, to certain *Cordaites*.

4. The persistence of the double transfusionary sheath in the true Pines of the Middle Cretaceous, although no similar structures have been found in numerous representatives of contemporary Araucarineae.

5. The wound-reactions of the older Cretaceous Araucarineae, referred by the author to the new subfamilies Brachyphylloideae and Araucariopityoideae, indicate a derivation of the ancestral Araucarians from an Abietineous stock.

6. The pitting of the older Araucarineae, which still survived in the Middle Cretaceous, showed a marked deviation from that found in *Agathis* and *Araucaria*, and a transition towards the type of pitting found in the Abietineae, while the oldest structurally known type of the Abietineae,

Prepinus, shows no tendency whatever towards the Araucarian type of bordered pits.

In this enumeration no account is taken of anything but sporophytic features. The gametophytes can more conveniently be considered at a later stage, when certain investigations have been completed.

Before summing up the results of the present research, it is necessary to say something in regard to the interpretation of transfusion tissue originally put forward by Worsdell and more recently elaborated by Bernard. In his article on 'Transfusion Tissue' (Trans. Linnean Society, London, vol. v, Part 8, Second Series), Mr. Worsdell makes the following statement: 'Transfusion tissue, which occurs almost universally in the leaves of Gymnospermous plants as an auxiliary conducting-system, has phylogenetically been derived from the centripetally-formed xylem of the vascular bundle, and is thus, morphologically, an integral part of the bundle itself.' The present research strongly supports the general accuracy of Mr. Worsdell's conclusions. It is, however, rendered doubtful if the elongated pitted elements found by this author on the ventral side of the protoxylem of the leaf-bundle in many Conifers can in reality be regarded as vestigial centripetal tracheids. It seems much more highly probable, from the conditions observed in *Prepinus* and in species of Cretaceous *Pinus*, that such elongated elements with bordered pits are in reality vestiges of the ancestral inner transfusional sheath, the real centripetal xylem having disappeared at too early a stage to be represented even vestigially in living Conifers. In any case it will not do to characterize, without qualification, the transfusion tissue which occurs ventrally to the protoxylem in many Coniferous leaves as centripetal xylem, as has been the tendency on the part of recent authors.

Summary.

1. A primitive Abietineous type, closely related to *Pinus* and strongly resembling superficially the *Leptostrobus* of Fontaine and the *Pinites Solmsi* of Seward, has been found in the Middle Cretaceous (Raritan or Upper Potomac) of Kreischerville, Staten Island, N.Y.

2. It is characterized by the possession of short-shoots or brachyblasts of a generalized type, which were deciduous; but bore numerous spirally arranged instead of few verticillate fascicular leaves. The sheath of these short-shoots more nearly resembled that found in the section *Strobus* and allied sections of *Pinus*, but the component scales were not deciduous as in the Soft Pines.

3. The leaves attached to the brachyblasts differed from the fascicular leaves of Pines in having their paired resin-canals continuous to the very base. The leaves further possessed well-marked centripetal xylem. About the foliar bundles was present a complicated double sheath of transfusion

tissue closely related to the centripetal wood and resembling that found in some *Cordaites*.

4. The name *Prepinus* is proposed for this type in the belief that it is the direct ancestor of *Pinus*.

5. Many of the true Pines of the Middle Cretaceous possessed the same double transfusionary foliar sheath as is found in *Prepinus*, but entirely lacked the centripetal wood which is characteristic of that genus.

6. The elongated pitted elements described by Worsdell and others on the ventral side of the protoxylem in existing Coniferous leaves appear rather to be relics of the inner transfusion sheath, which is a feature of Cretaceous Pines, than of true centripetal xylem.

7. The Abietineae are the oldest living family of the Coniferales.

8. *Pinus* is the oldest living representative of the Abietineae, and has in all probability been derived from *Prepinus*, which shows many archaic features.

In closing, the writer wishes to offer his best thanks to Dr. Hollick of the New York Botanic Garden, in whose company most of the material here described was collected. He also wishes to express his indebtedness to Professor F. W. Oliver, of the University of London, for the opportunity of examining unique preparations of Cordaitean leaves.

DESCRIPTION OF PLATES XIII AND XIV.

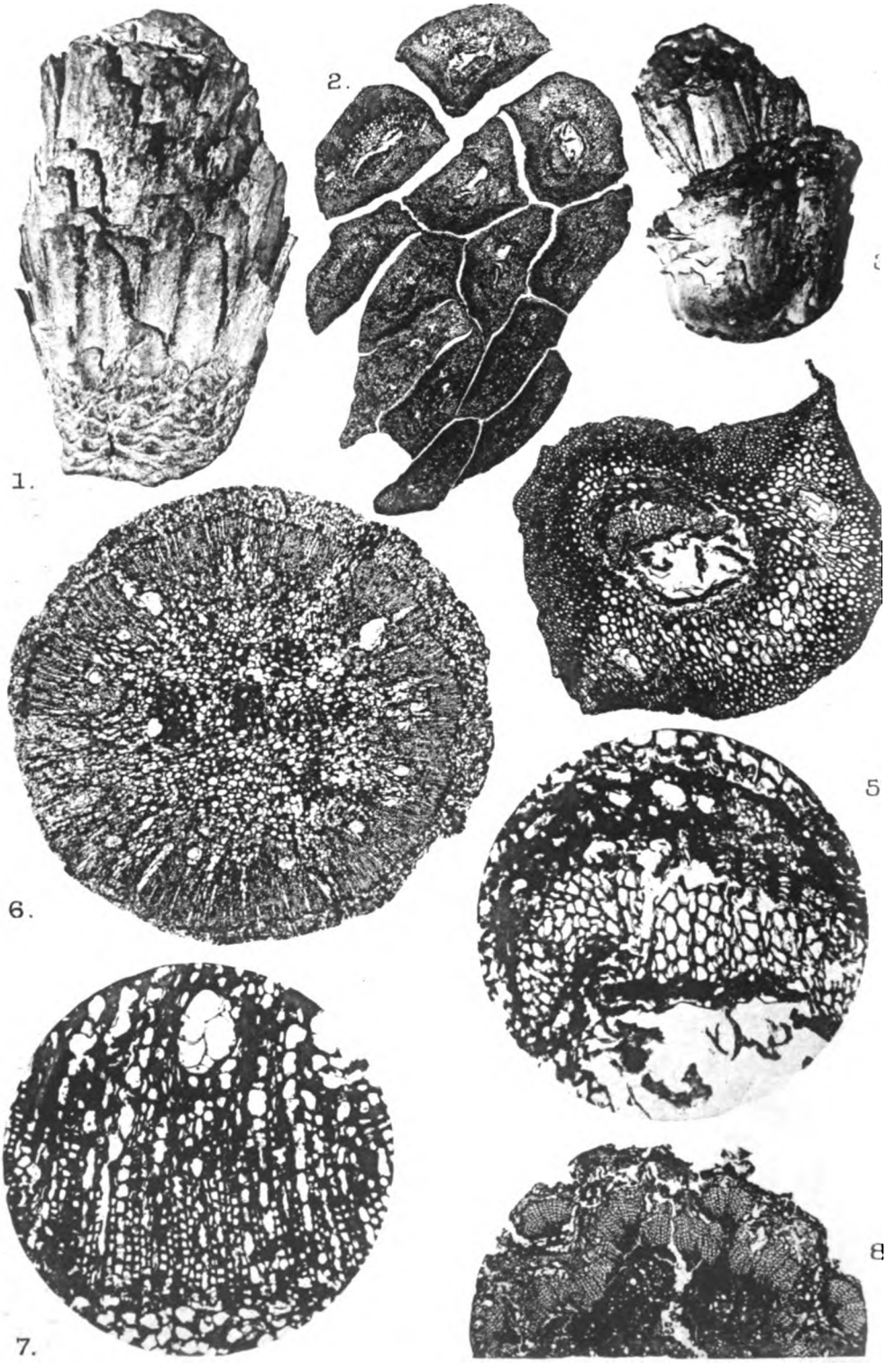
Illustrating Professor Jeffrey's Article on the Leaf of Cretaceous Pines.

PLATE XIII.

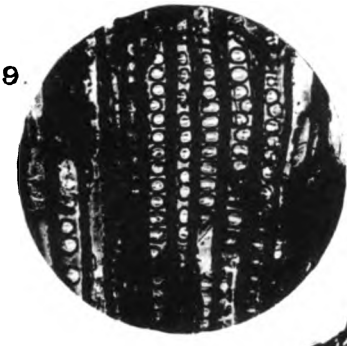
Fig. 1. Surface view of a short-shoot of *Prepinus statenensis*. × 10.
Fig. 2. Transverse section of fascicular leaves of *Prepinus statenensis*. × 13.
Fig. 3. Surface view of short-shoot of *Prepinus statenensis*. × 10.
Fig. 4. Fascicular leaf of *Prepinus statenensis*. × 40.
Fig. 5. Leaf-bundle of attached fascicular leaf of the same. × 100.
Fig. 6. Axis of the brachyblast of *Prepinus statenensis*. × 18.
Fig. 7. Part of the wood of the brachyblast of *Prepinus statenensis*. × 180.
Fig. 8. Upper part of the woody axis of a brachyblast of *Prepinus statenensis*. × 20.
Fig. 9. Tracheids of axis of brachyblast of *Prepinus statenensis*. × 180.
Fig. 10. The same. × 500.
Fig. 11. Detached leaf of *Prepinus statenensis*. × 64.
Fig. 12. The same, longitudinal section of the xylem. × 400.
Fig. 13. Detached leaf of *Prepinus statenensis*. × 30.
Fig. 14. Bundle of *Prepinus statenensis* in transverse section. × 140.
Fig. 15. Part of the same. × 500.

PLATE XIV.

Fig. 16. Leaf probably of *Cordaites principalis*. × 180.
Fig. 17. Leaf-fascicle of *Pinus tetraphylla*. × 32.
Fig. 18. Leaf of *Pinus spc.* × 40.
Fig. 19. Leaf of *Pinus spc.* × 40.
Fig. 20. Leaf of *Pinus spc.* × 40.
Fig. 21. Leaf of the same in longitudinal section of bundle. × 180.
Fig. 22. Leaf of *Pinus spc.* × 40.
Fig. 23. Leaf of *Pinus spc.* × 40.
Fig. 24. Leaf of *Pinus spc.* × 33.



JEFFREY — CRETACEOUS PINE LEAVES.



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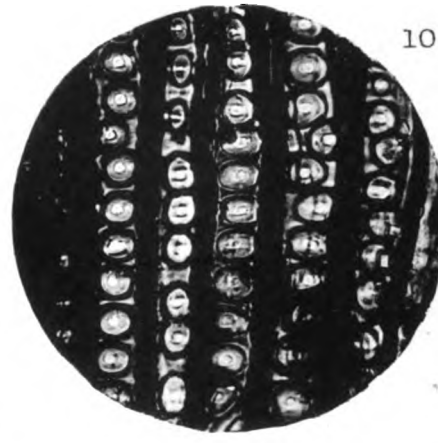

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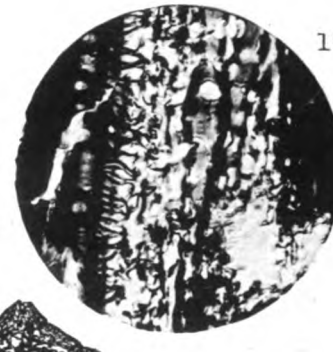

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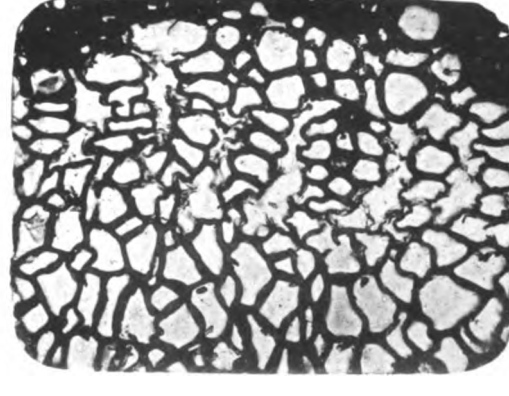

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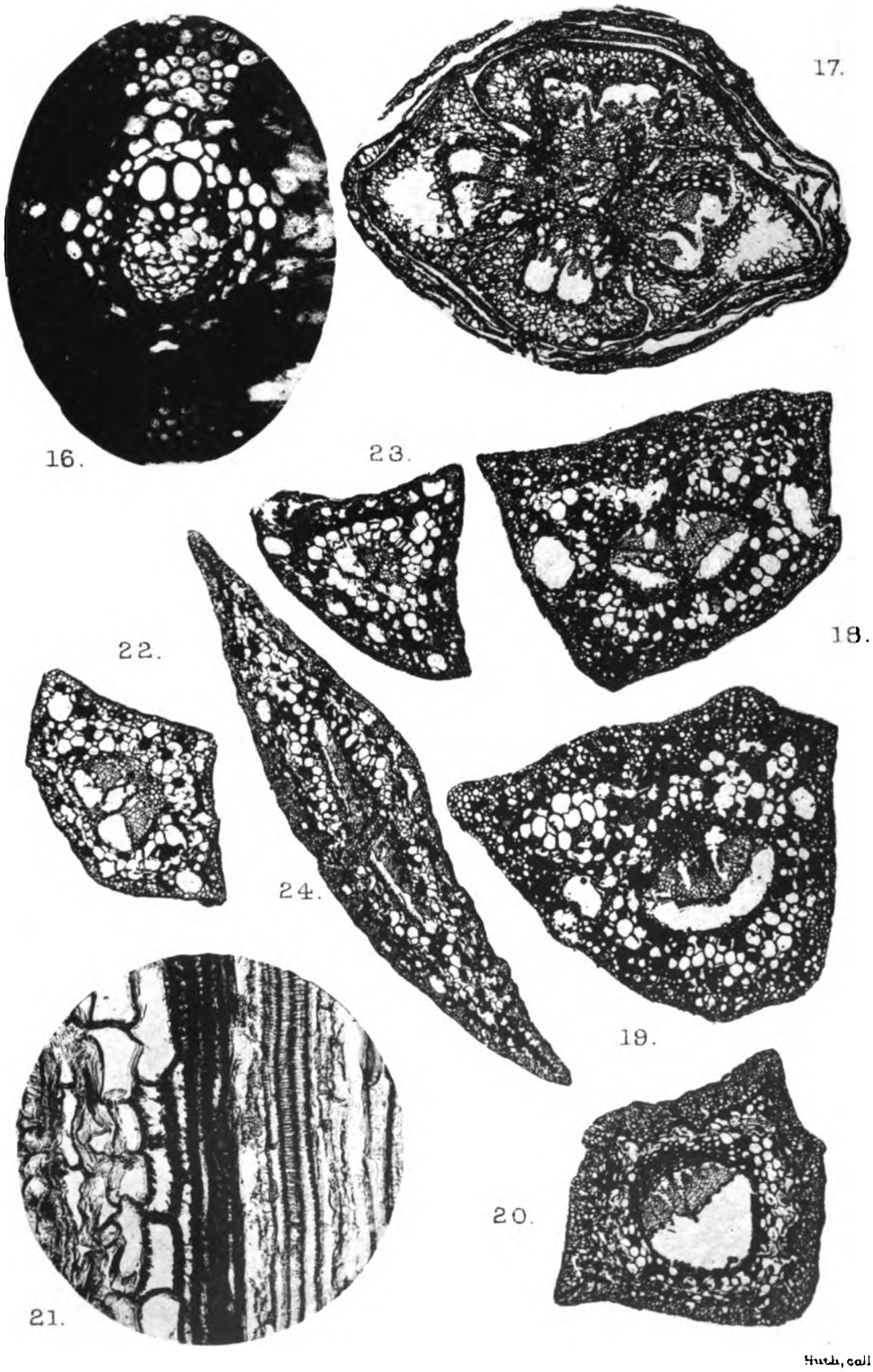

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JEFFREY — CRETACEOUS PINE LEAVES.

A Stigmaria with Centripetal Wood.

BY

F. E. WEISS,

Professor of Botany in the University of Manchester.

With Plate XV.

ONE of the most striking features in the structure of the Stigmarian axis is the fact that its primary wood shows, as a rule, a centrifugal development, while in the stems of *Lepidodendron* and *Sigillaria*, which formed the aerial continuation of the Stigmarian axis, the primary wood was invariably developed in a centripetal direction.

There seems to be a general opinion that this latter course of development of wood is the more primitive one. Its occurrence in the stems of the small number of recent Lycopodiales must perhaps not be taken as an argument in favour of this view; but the existence of this type of wood in the Lower Carboniferous *Calamites pettycurensis*, in *Sphenophyllum*, and in the Pteridospermeae, where the 'old wood' may be taken as a precursor of the 'new wood' of the Cycadales (see Scott, '02), are facts which seem to indicate the course taken in the evolution of the vascular system in the stem of the higher plants.

Whether the centrifugal development of the wood in the Stigmarian axis is the primitive condition of that organ, or similarly due to a substitution of 'new wood for old', has, however, remained an open question.

It is true a few specimens of *Stigmaria* have been described with a centripetal development of their primary wood, but this character seems in no way associated with other primitive characters. On the contrary, the *Stigmaria Brardii* described by Renault ('88) in the first instance as *Stigmaria flexuosa*, from Tracy St. Loup, near Autun, which, in its outward appearance, resembled the *Stigmaria rimosa* of Goldenberg ('58) (Pl. XII, Fig. 3), should, according to Solms-Laubach ('94), be correlated with *Stigmariopsis*, which must probably be regarded as more highly specialized than *Stigmaria*. According to Grand'Eury *Stigmariopsis* is the basal portion of *Clatharia* and *Leiodermaria*, forms of *Sigillaria*.

As far as I know, no specimen of *Stigmaria* with centripetal wood has been described from the English Coal Measures, and as some importance is attached to this arrangement, both as regards the primitiveness of its

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character and the morphology of the Stigmarian axis, it seems desirable to describe in some detail a specimen which has come into my possession through Mr. James Lomax, of Bolton.

It was found in a calcareous nodule from the well-known Hard Beds of Halifax, and is of small size, the central vascular cylinder measuring only ·75 cm. in diameter. Dr. Scott, to whom I showed the specimen, drew my attention to the resemblance of its primary wood to that of the stem of *Lepidodendron mundum*, and particularly of the axis as a whole to that figured by Williamson ('89) as a stem of *Lepidodendron mundum* with secondary thickening. An examination of this specimen in the Williamson collection at the British Museum convinced me that we had to do with the same plant as that described by Williamson, and the account which follows will, I think, suffice to show that in it we have a true Stigmarian axis. Whether or not it is to be associated with the stem showing the structure of *Lepidodendron mundum*[1] will remain for subsequent discussion. It is interesting to note that the specimen described by Williamson (cabinet number 416 b.) comes from the same locality as the one before us, and the agreement with our specimen is so close that I was at first inclined to believe that they must have been cut from the same block, but Mr. Lomax assures me that the nodule from which the second specimen was cut was quite untouched when he commenced to work on it. How many sections the first nodule yielded it is impossible to say, but one preparation in the Cash Collection, now in the University of Manchester, is obviously from the same block, and is also labelled *Lepidodendron mundum.* (Cash Collection, Q. 403 A.)

It should also be mentioned that there are in Dr. Scott's collection several slides of an axis, slightly larger but somewhat compressed, which appear to be sections of a similar plant (Nos. 1815 and 1825).

The vascular axis of the recently discovered specimen is about the same size as Williamson's specimen ; and seen in transverse section might well be mistaken for a *Lepidodendron* twig. Of the outer tissues the periderm alone is preserved, and is arranged in wide folds around the vascular cylinder (see Fig. 1, Pl. XV). In its greatest extension it measures 4 cm., but this does not give a real idea of its size, as one of its folds comes right in to the central cylinder. As near as possible the periderm must have had a circumference of not less than 15 cm., while that of the central cylinder is about 2 cm.

The *periderm* consists of 6–8 rows of thin-walled rectangular cells, and is very similar to that of a typical *Stigmaria*, such as *S. ficoides*. It shows a considerable tangential extension of many of the external rows of cells, which has been recognized as a characteristic of the Stigmarian bark. (See Scott,

[1] According to Mr. James Lomax the stem showing internally the structure of *Lepidodendron mundum* had externally the marking of *Bothrodendron*.

'Studies in Fossil Botany,' p. 227.) The layers of cells, which must have clothed the periderm on the outside (outer cortex), whether a Stigmarian or Lepidodendroid axis, have entirely disappeared: only in one or two places does one find remains of small rounded cells of the nature of a primary cortex. They are much compressed, and their very poor preservation points to their having been of soft and yielding character, like the outer cortex of a *Stigmaria*, and not of the hard dense nature one finds in the aerial stem of a *Lepidodendron* or *Sigillaria*, and particularly of *Bothrodendron*. Here and there a somewhat denser group of primary cells has been preserved, and these evidently represent the remains of a rootlet-cushion. The presence of these rootlet-cushions, taken in conjunction with the general absence of a resistant outer cortex, the very extensive circumference of the periderm as contrasted with the small diameter of the vascular axis, and the peculiar structure of the periderm cells, all go to prove the Stigmarian nature of the specimen. Of the rootlets which it bore, nothing definite can be said, as none are found in continuity with the specimen. The numerous rootlets, which surround the axis, are not of any one particular type, and they may, of course, belong to other neighbouring Stigmarian axes. Indeed the very bad preservation of the softer tissues of the specimen before us would seem to indicate that it had been dead long before it became petrified, and was invaded by the rootlets of surrounding plants. As is usual in Stigmarian axes, the tissues lying between the periderm and the vascular cylinder are very defective. One would indeed not expect them to be well preserved in a specimen in which the outer cortical tissues were destroyed. As a matter of fact the space within the periderm has been largely invaded by Stigmarian rootlets, and only here and there are found dense brown masses, consisting probably of the débris of the delicate middle cortex. In certain places there are more or less irregular patches of wide and short reticulate tracheids (see Fig. 7, Pl. XV), which seem to have some connexion with the rootlet bundles, in connexion with which they will be referred to again. It is interesting to note that similar reticulate cells were described by Renault ('93)[1] in the cortical region of *Stigmaria Brardii*. As figured by him, however (Pl. XXXIX, Fig. 14), they appear more fusiform, as he indeed describes them to be, and they were found according to his description among the periderm cells, and not in the inner parenchymatous zone (middle cortex), which seems to be their position in the specimen under consideration. Indeed they are not only found in the outer portion of the middle cortex, but are present in large numbers close up to the vascular cylinder. This position may not be quite a normal one, as these tracheids have no doubt been displaced owing to the decay of the softer tissues, but there can be no doubt that they were associated with the vascular bundles within the soft, middle-cortex region.

[1] p. 195.

This region was, of course, traversed by the rootlet bundles on their outward passage; but, owing to the defective condition of the parenchymatous tissue, the direction taken by the rootlet bundles cannot be determined with any degree of certainty. They appear in this specimen as irregular in position as those figured by Williamson ('87) in *Stigmaria ficoides* (Plate XII, Figs. 39–41). In all probability they ran somewhat obliquely in this zone, while close to the vascular cylinder their course, as is usual in *Stigmaria*, was almost parallel to the Stigmarian axis. In this region, therefore, the rootlet bundles are cut almost transversely (see Fig. 5, Pl. XV), and they will be seen to consist of a group of medium-sized tracheids radiating very regularly from a centre of smaller elements. The very regular arrangement of the tracheids is suggestive of secondary growth having taken place in these rootlet bundles, a condition which is common in *Stigmaria*, and the fact that the bundles have attained different dimensions (see Fig. 5, Pl. XV) is in favour of this view. But, while in *Stigmaria ficoides* the secondary growth is only on the outside of the monarch rootlet bundle, here the secondary growth has taken place evenly all round the primary elements. Vascular bundles showing similar centric arrangement have been described and figured by Renault ('93) (Pl. XXXIX, Fig. 11) in the case of *Stigmaria Brardii*, with which our *Stigmaria* agrees also in the possession of the above-described reticulate tracheids. In *Stigmaria Brardii*, however, these centrically arranged bundles were the exception rather than the rule, and were taken by him to be arrested branches of the Stigmarian axis rather than the rootlet bundles, which he considered to have secondary wood, arranged in fan-shaped manner only on the outside of the primary wood. In our *Stigmaria*, on the other hand, the cyclic arrangement is the normal one and is evidently that of the rootlet bundles. The primary elements are so small and so few in number that it is impossible to distinguish proto- and metaxylem, but the general impression gained is that of a mesarch bundle, which view is supported by the appearance of the rootlet bundles in their course through the secondary wood (see Fig. 4, Pl. XV).

Owing to the state of preservation there is very little remaining of the phloem and cambium regions. In radial longitudinal sections a few rows of elongated parenchymatous cells are found on the outer boundary of the secondary wood, but they afford very little clue as to the nature of these tissues.

The *secondary wood* (see Fig. 2, Pl. XV), on the other hand, is fairly well preserved, and of normal type. Its nature and extent agree with that of the specimen described by Williamson ('89) as *Lepidodendron mundum* (see his Fig. 15, Pl. V). It consists of medium-sized tracheids, ·04 to ·05 mm. in diameter, arranged in regular radiating rows, and showing only slight irregularity, where new rays of wood elements become added as secondary growth proceeds. The tracheids are square in transverse section, and are of

the scalariform type, the strong horizontal bars of the lignification being connected by fine vertical striae, as has been noted and figured by Williamson ('89) for *Lepidodendron mundum*, as well as for other *Lepidodendra*.

We come now to the most important feature of this *Stigmaria*, and that is to the *primary wood*. As mentioned above, this differs in essence from that of other *Stigmariae* in being centripetal in its development instead of centrifugal. Towards the centre of the axis the secondary tracheids become considerably smaller, without losing anything of the regularity of their arrangement. Then within the cylinder of secondary wood we come to a very definite primary wood, beginning with small protoxylem elements on the outside, and followed on the inside by a metaxylem consisting of about two rows of tracheids, the innermost of which are of very large dimensions. In radial sections the narrow elements at the outside of the wood exhibit often spiral and annular markings, and no doubt this region consists of the protoxylem elements. The metaxylem, on the other hand, consists of scalariform tracheids, some of which are twice the size (·075 to ·09 mm.) of the largest elements of the secondary wood. It was no doubt the general arrangement of the metaxylem, as well as the small number and the large size of its tracheids, which influenced Williamson ('89) in his identification of the axis with secondary wood figured by him [1] with *Lepidodendron mundum*, with which the primary wood shows such singular agreement.

Within the metaxylem we come to a pith consisting of parenchymatous cells of rectangular shape when seen in longitudinal section, just as those one finds in *Lepidodendron mundum*.

The secondary wood is traversed by numerous parenchymatous rays, generally one cell thick, though occasionally two cells in thickness. Their thinner tissue has undergone some disorganization, so that they appear on the transverse section more like gaps, but they can readily be seen on the tangential longitudinal section (Fig. 4, Pl. XV). But a feature which distinguishes this type of *Stigmaria* from *Stigmaria ficoides* and some other forms is the absence of the very broad medullary rays which usually break up the woody cylinder into distinct wedges, and in which the rootlet bundles pass out to the exterior. This is also a character of the *Stigmaria* with centripetal wood, figured by Renault. The absence of these broad rays may very well have inclined Williamson to the belief that he was dealing with a Lepidodendroid branch. A close examination of the innermost portion of the secondary growth reveals the fact that there is a tendency of the cylinder of secondary wood to break up into segments, as is characteristic of *Stigmaria*. This is seen in Fig. 2, Pl. XV, particularly on the upper edge of the primary wood. In radial longitudinal sections, moreover, the rootlet bundles can be seen taking a horizontal course near the middle of the secondary wood, like that figured by Williamson ('87) for

[1] Fig. 15, Pl. V.

Stigmaria ficoides. In their origin these rootlet bundles will be seen to agree with those figured by Renault ('93) in a *Stigmaria* from the Champs des Borgis, near Autun (see Renault, Pl. XL, Figs. 8 and 9). This *Stigmaria*, like the *Stigmaria Brardii*, has centripetal wood. The rootlets in coming off did not break the ring of primary wood, but carried with them mainly the protoxylem elements.

Towards the outside the bundles become more oblique, as can be seen from the one included in Fig. 2, Pl. XV of the present communication. This oblique course of the rootlet bundles obviously prevents the cylinder from being split up in the manner usual in *Stigmaria ficoides.* Renault's *Stigmaria Brardii* seems to agree with our *Stigmaria* in this particular too. In tangential longitudinal section the rootlet bundle near the centre will be found to be cut transversely, and then, as stated above, will generally appear to have the smallest elements at the centre, surrounded by larger elements, possibly metaxylems (see Fig. 4, Pl. XV). This mesarch arrangement of the bundle, due, no doubt, to the inclusion of some of the centripetal primary wood of the axis from which the rootlet starts, prevents the bundle from having that tongue-shaped appearance in tangential section which is so generally characteristic of *Stigmaria,* where the primary xylem is centrifugal. The rootlet bundle is, however, generally connected with the secondary wood by special tracheids running down to it, as seen in Fig. 4. In these particulars our *Stigmaria* agrees with that from the Champs des Borgis, described by Renault ('93). In some cases the appearance is more normal, and the protoxylem seems to have metaxylem developed only on its outer sides (see Fig. 3, Pl. XV). The difference in these two arrangements would, no doubt, depend on the amount of primary wood which takes part in the formation of the rootlet.

On leaving the central woody cylinder, the lateral bundle always shows the centric arrangement so characteristic of this plant, and resembling the condition found occasionally by Renault in *Stigmaria Brardii* (see Fig. 5, Pl. XV). Immediately to the outside the bundles show a connexion with the wide reticulate cells which occur in large masses, though whether continuous in the living condition of the plant and forming an interlacing network like those of *Stigmaria Brardii* cannot be ascertained, owing to the defective state of preservation of the cortical layer in which these cells lay. By comparison with the similar cells of *Stigmaria Brardii* it seems likely that the reticulate cells formed a network in the cortical region. They were evidently connected with the vascular bundles, as can be seen both when these latter are cut transversely (Fig. 6, Pl. XV) or when a longitudinal view of them is obtained (Fig. 7, Pl. XV). In the latter case the vascular bundle is curiously constricted at intervals, not so much by a narrowing of the outer reticulate tracheids as by a constriction of the inner elements. Similar constrictions are shown in the leaf-trace of the Lepidodendroid stem

from the calciferous sandstone of Dalmeny, described by Seward and Hill ('99). In Fig. 21, Plate III of their memoir, such a constricted bundle is seen lying, applied there to the secondary wood, which seems almost to take the place of the reticulate cells of our *Stigmaria*. Indeed, the tangential view of the inner portions of the secondary wood in Fig. 23 of Seward and Hill's memoir very closely resembles these tracheids. Whatever the functions of these reticulate tracheids may have been in this case, we may well institute a comparison between them and the transfusion tracheids met with in the leaves of the Lepidodendraceae and in the Coniferae, where, as in *Torreya* and some other forms, they have, according to Bernard ('04), reticulate markings. But a comparison with a leaf-trace does not upset the conclusion that these are rootlet bundles, for tracheids corresponding with transfusion cells, though differing in function, are found in the rootlets of *Stigmaria*. (See Weiss, '03.)

Traces of the vascular bundles may be seen, though somewhat indistinctly, making their way through the periderm layer to the exterior. Fig. 8, Pl. XV, represents one of these cases from a longitudinal section, showing a vascular bundle (*v. b.*) passing out accompanied by a strand of small parenchymatous cells. This, which undoubtedly represents a parichnos strand, i. e. a portion of the mid-cortex, passing through the periderm, does not preclude the identification of the axis as of Stigmarian nature, for, as I have shown elsewhere (Weiss, '07), parichnos strands are not found exclusively in connexion with the leaf-trace bundles but also with rootlet bundles. And this I am satisfied is the character here, as the parichnos strand narrows down considerably to the outside, where the rootlet cushion would be found. Unfortunately the external tissues are very badly preserved, and it is difficult to identify the external process, though I believe it to be a portion of a rootlet.

It remains for me to sum up the arguments in favour of regarding the specimen under consideration as a Stigmarian axis rather than a stem of *Lepidodendron mundum*, as Williamson had concluded from a more fragmentary and less well-preserved specimen.

In the first place the very wide periderm (see Fig. 1, Pl. XV) and its peculiar structure (Fig. 8, Pl. XV), together with the remains of what must, I think, be taken to be rootlet cushions, speaks in favour of the Stigmarian nature of these tissues; while the absence of primary outer cortex of hard texture distinguishes it from the *stem* of *Lepidodendron mundum*.

In the curious centrical lateral bundles (Fig. 1, Pl. II) and in the possession of a system of delicate reticulate cells (see Figs. 6 and 7, Pl. XV) this axis agrees more closely with the *Stigmaria Brardii* of Renault ('93) than with any Lepidodendroid stem, not excepting the possible *Lepidophloios Harcourtii* from Dalmeny, described by Seward and Hill ('99). It must be remembered, however, that the reticulate cells of the French specimens

appear to be in the periderm and not in the middle cortex as in the *Stigmaria* under consideration.

On the other hand, the course of the lateral bundles through the secondary wood is, in some respects, more like that of a leaf-trace than of a rootlet, though its connexion with the secondary tracheids through which it passes is in keeping with the Stigmarian rootlets (see Figs. 3 and 4, Pl. XV). The bundles, in their origin and course, agree, too, very nearly with those of the *Stigmaria* from the Champs des Borgis, Autun, described by Renault ('93). There remains to be taken into consideration the arrangement of the protoxylem with its obvious centripetal development. Seeing that Renault has described some undoubted instances of Stigmarian axes with centripetal wood, this arrangement, though more characteristic of Lepidodendroid stems than Stigmarian axes, must not weigh too much against the above-mentioned structure. In fact, its occurrence in conjunction with the peculiar centric lateral bundles and the reticulate tracheids goes far, in my mind, to establish, not an identity but a correspondence, between the axis under consideration and the *Stigmaria Brardii* of Renault. I cannot consider them as identical, for the amount of primary centripetal wood and the wavy outline of the latter in *Stigmaria Brardii* obviously distinguish it clearly from our specimen. The same may be said of the other *Stigmaria* with centripetal wood described by Renault from the Champs des Borgis, and with which our *Stigmaria* does not entirely agree.

On the other hand, the very close agreement of the primary wood of our *Stigmaria* with that of the *Lepidodendron mundum* (now identified with *Bothrodendron*) suggests the correlation of these two plant-remains.

While believing the specimen to be of Stigmarian character, I would agree so far with Williamson in considering that it might very well belong to the same plant (*Bothrodendron*) to which the leaf-bearing axis described under the name of *Lepidodendron mundum* belongs. This latter is generally of small dimensions, and all specimens, except for the single one described by Williamson, and which I consider identical with the *Stigmaria* now under consideration, have been devoid of secondary thickening. This need not, however, be considered an obstacle, for in the first place the fragments so far described may be secondary branches, while, even should they be main shoots, it is quite conceivable that secondary growth may have only taken place in the basal region of the same, and have therefore only been of limited occurrence. Such an occurrence has been described for *Psilotum* by Boodle ('04) and by Miss Ford ('04). Should the same apply to *Bothrodendron*, the secondary growth might occur either at the base of the aerial axis or in the Stigmarian region, or more probably in both.

I incline to the opinion that the axis described in the preceding pages is, as stated above, the Stigmarian portion, both from the very wide nature of the cortex, and from the peculiar lateral bundles, which differ materially

from those known in the upper part of the plant, and which are associated with the system of reticulate tracheids not found in the stem but known to us in other Stigmarian axes. Their function in the extensive cortex of the axis cannot, I think, be that of transfusion tracheids, but rather that of water-storage.

It might be thought that this is not a function likely to be required by a plant rooted, as far as we know, in a marsh; but we know that many plants growing in fresh-water marshes—and still more so in salt marshes—are of xerophytic habit. It must also be remembered that the Lepidodendraceae show certain xerophytic characters, and the Stigmarian rootlets were possibly not very efficient organs for rapid absorption, so that a means of storing water at the base of the stem may have been of some service to the plant in regulating the water supply during certain periods.

That such a tissue occurred in some other forms of *Stigmaria*, as shown by Renault, would, I think, indicate that it is no individual peculiarity of this species, but that it serves some more or less important function of this part of the plant.

In conclusion I should like to mention that, besides the similar *Stigmarias* referred to above as in Dr. Scott's collection, there are one or two other axes, both among Dr. Scott's preparations and among my own, which seem to be of Stigmarian nature, though possessing centripetal wood. The evidence in support of their Stigmarian character is, however, still insufficient, and I have therefore left them out of consideration for the present.

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EXPLANATION OF PLATE XV.

Illustrating Professor Weiss's Paper on *Stigmaria*.

Fig. 1. Transverse section of *Stigmaria*, showing the central cylinder with secondary thickening surrounded by the wide periderm layer (*per.*) which has been much compressed and is thrown into folds. The border of the periderm has been outlined with Indian-ink to render it more distinct. Slide R. 881 *b* magnified. × 2½.

Fig. 2. Enlarged view of central cylinder, showing the centripetal primary wood (*p. w.*) surrounded by mantle of secondary wood (*s. w.*); at the upper edge of the central cylinder a rootlet bundle is passing to the exterior. Q. 403 A. × 24.

Fig. 3. Tangential longitudinal section through the secondary wood, showing rootlet bundle (*r. b.*) on its way to exterior. R. 881 *i*. × 42.

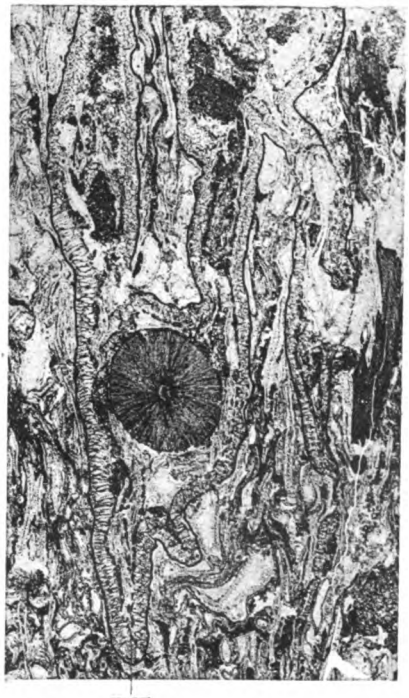
Fig. 4. Tangential longitudinal section through the secondary wood, showing the medullary rays (*m. r.*), and a rootlet bundle (mesarch) in the centre of the photograph. *s. r.* = scalariform tracheid. R. 881 *g*. × 46.

Fig. 5. Transverse section at the outer boundary of central cylinder, showing two lateral mesarch bundles (*r. b.*) with centric arrangement of secondary tracheids. The tracheids of the lateral branch are much smaller than those of the secondary wood (*s. w.*) of main axis. R. 881 *c*. × 100.

Fig. 6. Transverse view of lateral bundle (*v. b.*) in the region of middle cortex, showing its connexion with a patch of reticulate tracheids (*r. t.*). R. 881 *h*. × 100.

Fig. 7. Lateral vascular bundle (*v. b.*) in region of middle cortex, showing characteristic constrictions and connected with a large patch of wide reticulate tracheids (*r. t.*). R. 881 *g*. × 40.

Fig. 8. Portion of longitudinal section of periderm (*per.*) through which a vascular bundle (*v. b.*) is passing to rootlet cushion (defective). *par.* = parichnos strand of parenchymatous cell running below the rootlet bundle. R. 881 *i*. × 30.



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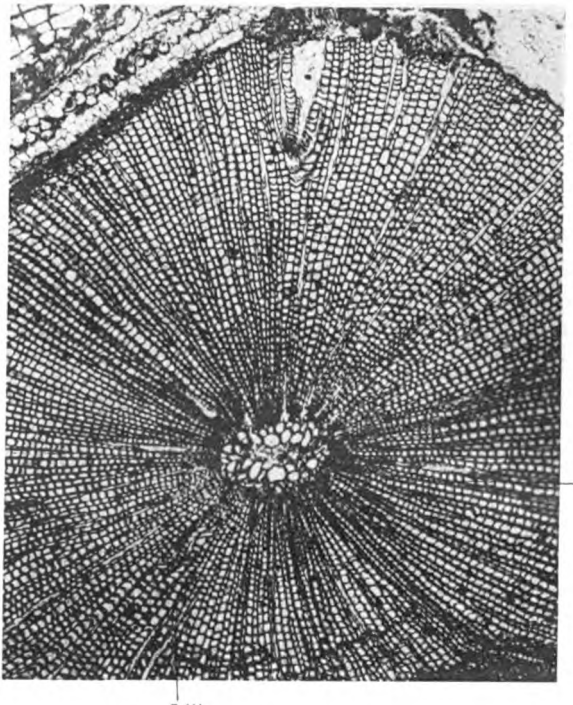

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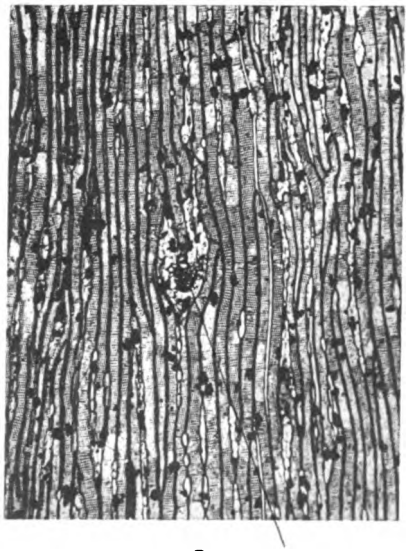

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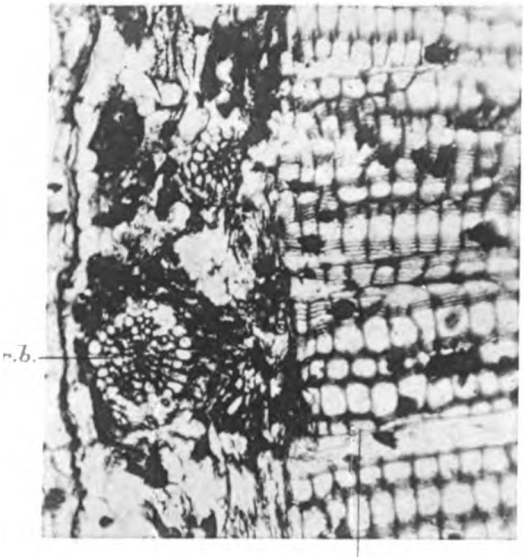
WEISS — STIGMARIA.



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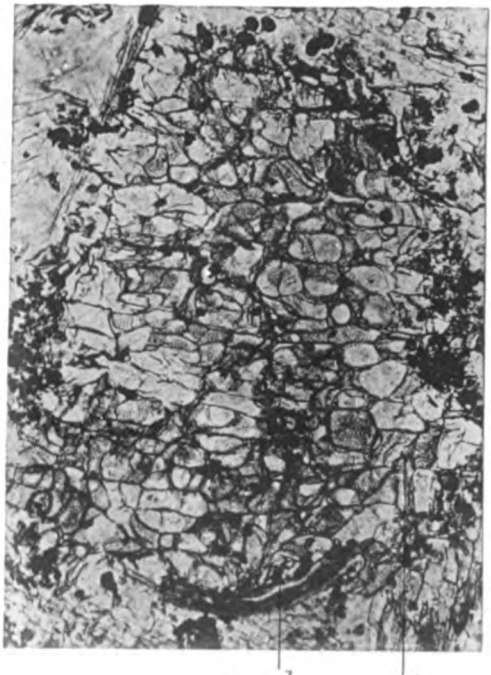

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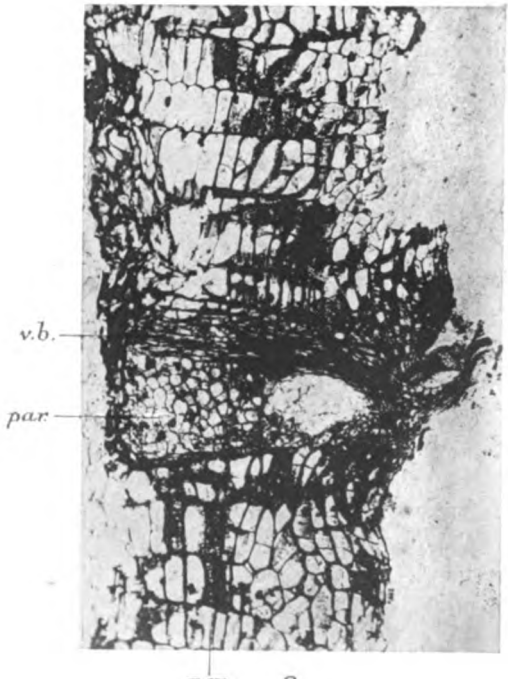

8.

Heath coll.

On the Production of Dwarf Male Prothalli in Sporangia of Todea.[1]

BY

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With Plate XVI.

WHEN examining sporangia of filmy species[2] of *Todea*, a few years ago, I was surprised to find that closed sporangia attached to the leaf sometimes contained antheridia, owing to the spores having germinated and at once formed the male organs. This appeared so interesting that I attempted by a series of experiments (in 1904 and 1905) to gain some light on the cause of this precocious formation of sexual organs. Such results as were obtained did not lead very far towards an explanation of the phenomenon, though determining its relation to certain conditions. Publication[3] was consequently postponed awaiting an opportunity of making further experiments. As, however, the phenomenon itself is of some importance, it seems advisable not to delay longer, but to place the observation on record with the results obtained, in the hope that other botanists may carry the matter further.

Todea Fraseri, Hook. et Grev. and *T. hymenophylloides*, Rich. et Less. were the species on which most of the observations were made, and, of these, *T. Fraseri* was found to be more ready to form intrasporangial antheridia. The mature sporangia are dark green owing to the dense chlorophyllous contents of the ripe spores, and ultimately appear greenish black by reflected light. It is in these very dark sporangia after they have passed maturity that antheridia are sometimes to be found. An example is given in Pl. XVI, Fig. 1, which is from a microtome-section of a closed[4] sporangium obtained from a fertile leaf of *T. Fraseri*. The spores are seen to have germinated and produced antheridia, eight of which are visible in this section. The details are better seen in Fig. 2, which represents the

[1] From the Jodrell Laboratory, Royal Botanic Gardens, Kew.

[2] The filmy species of *Todea*, Willd. (cf. Synopsis Filicum) form the genus *Leptopteris* of Presl.

[3] A short preliminary account was read before Section K of the British Association at Cambridge in 1904.

[4] The injuries in the wall of the sporangium are due to the dragging of the razor.

[Annals of Botany, Vol. XXII. No. LXXXVI. April, 1908.]

product of germination of a single spore in the same sporangium. The antheridium at the top contains numerous sperm-cells (*s.*), and is borne by an extremely reduced gametophyte, enclosed at the base by the ruptured exosporium (*e.*). Below the cavity of the antheridium there are only five cells. Of these the upper two may be regarded as forming the basal part or stalk of the antheridium, thus leaving only three cells for the prothallus. No rhizoid-cell is present in this specimen.

In the sporangium shown in Fig. 1, nearly all the spores had produced dwarf male prothalli similar to Fig. 2, but in other cases the number of male prothalli in a sporangium was usually small. For a typical case, in which intrasporangial germination has taken place and advanced sufficiently, the contents of the sporangium may be described as follows: a few prothalli of about three cells and usually with a rhizoid; a few prothalli bearing antheridia, and nearly always without developed rhizoids; the majority of the spores showing no signs of germination. In sporangia in which only two or three spores had germinated, the young prothalli had no antheridia, and usually bore rhizoids. From the presence of rhizoids, and from comparison with experimental cultures, in which germination has not advanced very far, one may infer that the prothalli first formed are not those which finally bear antheridia.

The preceding remarks apply to sporangia attached to the leaf, the observations having been made on two plants of *T. Fraseri* growing in the Filmy Fern House at Kew [1]. In this species the production of antheridia inside sporangia attached to the leaf was observed in four different years, but was not seen on living plants of *T. hymenophylloides*, *T. superba*, Col. or *T. Moorei*, Baker. A few young prothalli were several times found in *T. hymenophylloides*, and occasionally in the two other species, among the spores in closed sporangia, but these prothalli did not bear antheridia, and were usually not far enough advanced to do so.

A short description of experiments may now be given. Pinnae or pinnules bearing dark green sporangia were removed from the living plant, and, while kept damp, were examined under a low power of the microscope, to see that none of the sporangia had burst [2]. The contents of some of the oldest sporangia were then examined, and, if germination had not begun in these, the other sporangia were taken as suitable for experiment.

The essential feature in a number of the experiments consisted in keeping free spores and closed sporangia under similar conditions, and then comparing the mode of germination of the spores in the two cases. The free spores were taken from sporangia on the same leaf (usually on the

[1] At the time at which some of these observations were made, the temperature of this house was usually kept between 10° and 13° C., but the best cases of intrasporangial germination were found in the warmer months, when the temperature was often considerably higher.

[2] When damp, sporangia, which have dehisced, may close again sufficiently to render close examination necessary.

same pinnule) as the closed sporangia used in the same experiment, and the two cultures were placed side by side in a greenhouse, Petri-dishes being used in most cases. The temperature of the greenhouse varied considerably, but during the day-time, in some or most of the experiments, was usually between 16° and 21° C. One experiment including several cultures was set up as follows. Free spores were kept (1) on damp blotting-paper, (2) on damp sand, (3) immersed in water in a watch-glass. Three parallel cultures were made, using detached closed sporangia instead of spores. These were all exposed to daylight, and a similar set of six cultures was kept in darkness (in a light-tight box). To these were added pinnae bearing sporangia, and placed on damp blotting-paper in light and in the dark. This experiment was carried out both in *T. Fraseri* and in *T. hymenophylloides*, and some of the pairs of cultures were repeated two or three times.

The general result obtained was that dwarf male prothalli with antheridia were found in several of the sporangia, but were never produced by the germination of free spores. From an examination of the contents of the sporangia, it was evident that, under the conditions of the experiments, a certain number of dwarf prothalli with antheridia were formed in the sporangium whenever the germinative power of the spores was sufficiently strong, and the culture could be kept healthy long enough. Their formation is thus connected with germination inside the closed sporangium, and this again is due to excessive dampness, which prevents the sporangium from dehiscing when mature. This was shown by uncovering a Petri-dish containing a pinna with sporangia on damp blotting-paper. A few minutes' exposure to dry air, if the culture was not too damp, was sufficient to cause normal dehiscence of several sporangia.

Figs. 3 and 4 are examples of dwarf gametophytes from closed sporangia, which had been kept damp in the light for twenty-one days. They are shown in optical section, with the cell-contents omitted (as in most of the figures), *a.* being the cavity of the antheridium. It will be seen that Fig. 3 is very similar to Fig. 2, described above, but differs in the presence of a rhizoid-cell (*r. c.*), while Fig. 4 has no rhizoid-cell, and is exceptional in having a rudiment of a lateral branch (*l.*), which, from comparison with another specimen, may have been destined to form a second antheridium.

Sporangia which fail to dehisce behave alike when exposed to light, whether they remain attached to the living plant, or to a pinnule kept damp on blotting-paper, or whether they are detached and similarly treated; at any rate no marked difference in the germination of the spores was noticed. Under favourable conditions a number of spores germinate, and some of them produce dwarf prothalli and antheridia[1]. The prothalli

[1] In the case of sporangia immersed in water, germination was usually not very satisfactory, perhaps owing to defective aeration.

appear to be incapable of bursting the wall of the sporangium, and they and the ungerminated spores ultimately die. Several attempts were made to obtain motile spermatozoids, but always failed. Sporangia were ruptured, and the contents examined in water, but usually any antheridia present refused to burst. Once or twice an antheridium was seen to dehisce, and the spermatocytes passed out, but the spermatozoids did not even uncoil.

Free spores, when germinated in the light, formed normal prothalli (see Fig. 11), which, however, showed some differences according to the nature of the culture. On sand the primary rhizoid was usually long; on damp blotting-paper it was often well developed, but sometimes rudimentary or absent; in water it was often rudimentary or absent, though occasionally fairly well developed. On blotting-paper reduction of most of the rhizoids was sometimes observed, and appeared to be caused by wetness of the culture. In water the prothallus tended to be more rounded, or thicker and less elongated, than on sand or blotting-paper.

In most cases free spores were found to germinate very readily in the light. Direct sunlight was kept off the cultures by a screen, and a blind on the top of the greenhouse reduced the light. From an experiment in which the light was further reduced and growth less rapid, one may assume that, if the illumination was above the optimum, it was not far above it [1].

The spores are also capable of germination in darkness, but growth is slower than in light, and finally comes to a standstill. Figs. 5 and 6 are from two cultures of *T. Fraseri* after twenty-six days in darkness. Fig. 5 is a prothallus and antheridium from a closed sporangium, and Fig. 6 is a prothallus formed by the germination of a free spore. In Fig. 5 the sperm-cells (*s.*) are shown in the antheridium, and the greater part of the prothallus is enclosed in the exosporium (*e.*), so that it cannot be seen how many cells are present. This experiment shows that light is not necessary to the production of antheridia inside the sporangium. Gametophytes with antheridia, similar to Fig. 5, were formed in several sporangia in darkness, and, just as in cultures in the light, they were accompanied by some small prothalli without antheridia. A rather common feature in sporangia kept in darkness is the presence of prothalli with abortive antheridia, and what may be described as vegetative transformations of young antheridia. These will be referred to again later. Fig. 7 is one of the most reduced gametophytes met with, and is from a sporangium after twenty-two days in darkness. The antheridium has a very small cavity (*a.*), below which there are only two cells, one probably representing the stalk, and the other the prothallus.

In *T. hymenophylloides* the germination of the spores and the growth of the prothallus are less rapid than in *T. Fraseri* under the conditions of

[1] Heim ('96, p. 354) found that for certain Fern-prothalli the strongest growth took place in daylight (Munich) reduced by 20–25 per cent.

the experiments, and it is probably in consequence of this, that fully formed antheridia were only rarely found in cultures of closed sporangia of *T. hymenophylloides*. In several cases the cultures became unhealthy before germination was sufficiently far advanced. Figs. 8 and 9 are prothalli from a closed sporangium after thirty-two days in the light. In Fig. 9 no antheridium has been formed, while in Fig. 8 an abnormal antheridium, in which the cavity (*a.*) contains chlorophyll, is borne by a very rudimentary prothallus. The presence of a rhizoid (*r.*) in this type of prothallus is not common. In Fig. 10 (also from a closed sporangium of *T. hymenophylloides*) the form of the cells indicates that the prothallium had made an abortive attempt to form an antheridium. Several similar examples were seen in *T. hymenophylloides* in closed sporangia. In these, two or more cell-divisions had taken place, cutting off cells similar to those which normally go to form the antheridium, but the cells generally retained their chlorophyll, and sometimes became enlarged.

Examples of well-advanced prothalli grown from free spores on blotting-paper in the light are given in Figs. 11 and 12. Fig. 11 is from a spore of *T. Fraseri* after thirty-six days. The rhizoid (*r.*) is rudimentary in this specimen, and, as often occurs when the rhizoid is reduced, its wall is thickened in the form of a cap at the apex. Fig. 12 is from a spore of *T. hymenophylloides* after thirty-two days, and may be compared with Figs. 8 and 9, which are prothalli from a sporangium in a culture of the same age.

Some experiments of a different kind may now be referred to. Firstly, germination of free spores in distilled water and tap-water[1] was compared, and it was found that the spores germinated more rapidly in distilled water. Figs. 13 and 14 are from cultures of spores of *T. Fraseri* in light after three days. In Fig. 13 (in tap-water) the spore has burst its exosporium (*e.*), but probably remains undivided, while in Fig. 14 (distilled water) the exosporium has been thrown off, and the young prothallus consists of three cells including a fairly long rhizoid. These specimens were grown in watch-glasses with the water about 2 mm. deep[2]. For the growth of the prothallus, distilled water has the advantage for some time, but in one experiment after fourteen days the prothalli in tap-water proved to have slightly outstripped those in distilled water as to length[3]. In distilled water the rhizoid is usually well developed.

Germination in 5 per cent. solution of cane-sugar gave slower growth of the prothallus than in tap-water, and the rhizoids were short.

A number of cultures were started in water in upright glass tubes (4 mm. diameter), but these need not be described further, as several of these

[1] Rather hard water.

[2] The rate of germination depends to some extent on the depth of the liquid, the relation being inverse.

[3] This refers to the length of the prothallus, excluding the rhizoid.

cultures were spoilt by Bacteria. In cultures made in watch-glasses, the spores and prothalli generally remained healthy for a long time. Bacteria sometimes appeared where a number of spores were crowded together, and the germination of these spores was thus prevented or checked; but, where the spores were more scattered, Bacteria were seldom to be seen. It should also be mentioned that a fungus, which Mr. G. Massee, F.L.S., kindly named for me as *Dendryphium effusum*, Bon., was sometimes found growing on the wall of the sporangium, both in cultures and on the living plants, but the presence or absence of the fungus appeared to have no effect on the germination of the spores in the sporangium.

Figs. 15–20 are given to show the characters or peculiarities of some young stages of prothalli. Figs. 15 and 16 are two prothalli of *T. hymenophylloidæs* at the three-celled stage, showing different arrangement of the cells, Fig. 15 being from a culture of spores in water in the dark, Fig. 16 from a sporangium in light. Fig. 17 is a more advanced prothallus of the same species from a spore grown in water in the light. Figs. 18–20 are prothalli of *T. Fraseri*, Fig. 19 being from a culture of spores on blotting-paper in darkness after thirty-three days. The cell marked with a cross contained very little chlorophyll. In cultures in the dark it was often noticed that the last-formed cell had scanty chlorophyll-grains, i. e. when the prothallus had reached a five- or six-celled stage. Under the conditions of the experiments it is probable that, at this stage, growth had nearly ceased, light being necessary for further development. Fig. 18 is a prothallus grown on blotting-paper in the dark. The primary rhizoid-cell has not been formed, but a secondary rudimentary rhizoid is present. Fig. 20 is a prothallus from a similar culture to the last, and shows an unusual form with transverse growth.

In the germination of the spores of *Todea Fraseri* and *T. hymenophylloides*, there is some diversity in the arrangement of the cells of the young prothallus, but the commoner types agree well with the same stages in *Osmunda cinnamomea* and *O. claytoniana* as figured by Campbell, and *Todea barbara* as figured by Luerssen. Thus Figs. 14, 16, and 19 on Pl. XVI correspond with Campbell's Figs. 12 *a*, 5 and 14 ('92, Pl. III), and Luerssen's Figs. 6, 13, and 19 ('74, Pl. XXIII). In rather later stages also the prothalli of the filmy species of *Todea*, while showing variation in breadth, &c., reproduced several of the forms figured by Campbell and Luerssen in *Osmunda* and *Todea*, including occasional examples showing an early forking of the prothallus.

We may now return to a consideration of the results of the experiments. Free spores germinate normally, and produce prothalli of considerable size without forming antheridia[1]. Hence the production of dwarf male

[1] At the conclusion of the experiments the largest prothalli consisted of more than twenty cells, but no antheridia had been formed.

prothalli[1] is not a character of the species, but proves to be connected with germination of spores in the closed sporangium. The conditions under which spores germinate in a sporangium probably differ in several respects from those under which a free spore germinates, e. g. as regards aeration, water supply, and supply of inorganic salts, but there are no data for estimating these factors[2]. One obvious difference, however, may be pointed out as probably important. The spores in the sporangium must germinate under pressure[3], which must increase as more spores germinate. We may perhaps assume (1) that the pressure prevents the prothalli from growing to the size that they would attain if free, and (2) that the result of this will be the accumulation of certain organic food-substances, which would normally be used or diluted in connexion with growth. High concentration of these substances may cause special nutrition of the protoplasm, and this may lead to the precocious formation of antheridia[4].

In experiments with *Oedogonium* Klebs ('96, p. 280) found that lack of nutritive salts (due to using only a small quantity of water for the culture) favoured the formation of sexual organs. In sporangia of *Todea* the spores may perhaps suffer from lack of salts owing to defective water-supply, but, that this factor alone will not explain the formation of dwarf male prothalli, is shown by the fact that free spores germinate normally in distilled water. The spores, moreover, probably contain a certain amount of inorganic reserves. Perhaps the most probable view for the spores of *Todea* is that any factor, which hinders growth without checking the accumulation of certain soluble organic food-substances, will favour the formation of sexual organs. External pressure (compression) would be one such factor, and severe scarcity of water[5] would be another. The result of checking growth may be illustrated by another experiment of Klebs ('96, pp. 295–6). A species of *Oedogonium*, which grew in soft water, showed vigorous formation of antheridia when transferred to an aquarium containing hard water, growth and division being much hindered in consequence of the amount of lime contained in the water.

In sporangia of *Todea* the absence of antheridia on the first-formed prothalli is perhaps explained by the pressure of the sporangial wall being still insufficient. The vegetative transformations of antheridia mentioned in the earlier part of this paper were observed in rather old cultures, and may

[1] In the present paper the term 'dwarf male prothalli' refers to extremely reduced forms comparable to Figs. 2 and 3.

[2] Hence any explanation must at present be a matter of pure speculation.

[3] An attempt was made to imitate this condition by growing free spores in water and in gelatine under pressure supplied by a column of mercury, but the experiments failed; the control cultures showed that the conditions were unsatisfactory.

[4] It is possible, on the other hand, that conditions other than pressure in the sporangium may directly favour the formation of antheridia, and hinder growth as a secondary result.

[5] Drought has been given as one of the causes of precocious formation of antheridia on prothalli by Woronew ('94, p. 177) and others.

have been due to a change in the conditions inside the sporangium, caused by the collapse of some of the prothalli, often observed in old cultures.

In many Ferns it is well known that, when spores are sown crowded, the prothalli remain small and produce antheridia only[1]. This is somewhat similar to the formation of dwarf male prothalli in *Todea*, but such extreme reduction of the prothallus as that shown by *Todea* has not often been observed. For the Osmundaceae, Luerssen ('74, p. 469) states that the antheridia often appear on very young prothalli, but in the only figure he gives of an antheridium on a complete prothallus the latter consists of twenty-six or more cells. Borodin ('68, p. 438) describes dwarf male prothalli in *Allosurus*; these will be referred to later. Schacht ('49, pp. 756, 787, Taf. v, Fig. 1) describes extremely small prothalli bearing antheridia in *Pteris serrulata*[2], and his figure shows a case in which only an antheridium and two other cells are present, viz. a short cell containing chlorophyll, and a rather well-developed primary rhizoid. Cornu ('74, p. 161) describes similar prothalli (of two cells and an antheridium) in *Nephrodium Filix-mas*. In these two cases the degree of reduction is similar to that found in *Todea*. Woronew ('94, p. 177) also describes the formation of antheridia on reduced prothalli (of three or four cells) under unfavourable cultural conditions.

Regarding the power of Fern-spores to germinate in the dark, the earlier statements are contradictory. Thus Borodin ('68, p. 432) and Kny ('72, p. 4) described light as necessary for germination, while Schelting ('75, p. 328), experimenting with spores of four species of *Anemia*, *Pteris*, and *Aspidium*, found that they all germinated in the dark. Beck ('78, p. 780), however, found that spores of *Scolopendrium vulgare* would only germinate in the light, and Woronew ('94, p. 176) failed to germinate spores of ordinary Ferns in darkness, but succeeded with those of *Pilularia* and *Marsilia*. Heald ('98, p. 43) found that in *Ceratopteris thalictroides* the spores refused to germinate in the dark at a temperature of 19° to 21° C., though germinating in light at this temperature, but that at 32° C. they germinated well in darkness. He regards the conflicting results of earlier authors as probably explained by assuming that unsuitable temperatures were used in some of their experiments. Heald only experimented with one species of *Ceratopteris* and one of *Alsophila*, hence it is unsafe to

[1] Goebel ('82, p. 198). Millardet ('69, p. 50), referring to prothalli bearing antheridia only, states that in one entire sowing of *Osmunda regalis* he could not find a single archegonium. Prantl ('78, p. 499) found that the production of archegonia was connected with meristematic growth of the prothallus, inasmuch as reduced prothalli with no meristem ('ameristic prothalli') bore antheridia, but never archegonia. He attributes the ameristic condition to insufficient nutrition, light or water (and especially the dissolved mineral salts), but sometimes refers it to the nature of the spore itself. In a later paper Prantl ('81, p. 753) showed that absence of nitrates in the nutrient solution conduced to the formation of ameristic prothalli. Sadebeck ('79, p. 166) points out that the tendency to dioecism ascribed to Ferns is explained in most cases by the fact that certain prothalli have remained ameristic.

[2] In this species dwarf prothalli bearing antheridia are also described by Atkinson ('94, pp. 15, 16).

generalize from his results, but his determination of the influence of temperature is important. Since the publication of Heald's work, other papers dealing with the same subject have appeared. Burgerstein ('01, p. 92) found light necessary to germination. Schulz ('02, p. 97) experimented with several Ferns, and found that the spores only germinated in the light, except in the case of *Ceratopteris thalictroides*, Hydropterideae and Ophioglossaceae. Life ('07, p. 121) did not succeed in germinating Fern-spores in the dark, even when the temperature was 30° to 33° C. In his experiments the spores were sown on leaf-mould. Laage ('07, p. 111, &c.) made numerous experiments, using Knop-solution (1 per cent. and other strengths) and several other liquids. In darkness he obtained germination of spores of *Osmunda regalis*, *Pteris aquilina*, *Scolopendrium officinale* (*S. vulgare*, Sm.), *Aspidium aculeatum*, and *A. Filix-mas*, but failed with *Asplenium lucidum*, *Alsophila australis*, and *Polypodium aureum*.

From a consideration of these numerous researches, which in one or two cases gave opposite results for the same species, it appears very probable that the failure of spores to germinate in the dark was due in some cases to an unfavourable temperature. With the most suitable medium and temperature (both of which may vary for different species) it is possible that most or perhaps all Fern-spores might be capable of germination in the dark; but this remains to be seen.

In *Todea Fraseri* germination took place in the dark in tap-water between 60° and 70° F. (16°–21° C.), but neither the optimum temperature nor the best medium was ascertained. The nutrition of the spores, when germinating in darkness, is apparently provided by a reserve of an oily substance[1]. Spores were crushed in 1 per cent. solution of osmic acid, and it was found that oil was more abundant in *T. Fraseri* than in *T. hymenophylloides*. This may explain the greater readiness with which spores of the former species germinate, which is specially noticeable in darkness. The oil is probably partially converted into a soluble carbohydrate (see previous foot-note), which supplies the osmotic strength of the cell-sap necessary for germination. Definite limits of temperature for germination in this and other species of Ferns are probably fixed by those necessary for the action or formation of an enzyme, which converts the oil into a soluble carbohydrate and other bodies.[2]

[1] Borodin ('68, p. 446) states that, on the germination of Fern-spores in the light, the oil appears to be transformed into starch. Schulz ('02, pp. 90, 93) observed the presence of oil in Fern-spores, and found in *Aspidium*, &c. (which he did not succeed in germinating in darkness), that the formation of starch began with germination in light. He obtained the same result with *Ceratopteris* in darkness. This probably indicates that a sugar is formed, and that, when it reaches its maximum concentration, germination begins.

[2] The physiological process may be quite similar to that described by Green ('90, p. 384, see also Czapek, '05, vol. i, p. 129 et seq.) in the endosperm of *Ricinus*, the reserve-oil being split up by an enzyme (lipase, formed from a lipozymogen present in the resting spore) into a fatty acid and glycerine, and the glycerine being at once converted into sugar.

For examples of intrasporangial germination in other Ferns we may quote the case of the Hymenophyllaceae, in which the spores sometimes undergo the first few divisions in the closed sporangia (Sadebeck, '79, p. 159). It is also interesting to recall a case described by Scott ('04, p. 18) of germinating spores in a fossil Fern-sporangium, especially as the specimen appears to be allied to the Osmundaceae. Here, however, the wall of the sporangium is broken, and it is uncertain whether dehiscence may have taken place before the germination of the spores. It may be a similar occurrence to that described in *Todea hymenophylloides* (Boodle, '00, p. 484), viz. the germination of spores in a ruptured sporangium, when dehiscence has been weak, so as only to eject a few of the spores, and probably this is also the explanation of the case figured by Atkinson ('94, p. 7, Fig. 12) in *Pteris serrulata*. In a second case described by Scott ('06, p. 170, Fig. 27) in a fossil Fern, the germination may well have taken place in the closed sporangium.

Borodin ('68, p. 438) states that he obtained a certain number of dwarf male prothalli by sowing spores of *Allosurus sagittatus* in water in the light, and transferring them into darkness after 5–6 days (i. e. just when germination was beginning). The culture afterwards showed that most of the spores had remained unchanged, but that a certain number had produced dwarf gametophytes. The prothallus was reduced to a single cell, and bore from one to three antheridia. It is just possible that these reduced prothalli, and some of those described by Schacht and others, may have really been produced by previous germination in the sporangia from which the spores were taken for sowing.

The fact that in filmy species of *Todea* a certain degree of dampness can, by preventing dehiscence of the sporangium[1], cause extreme reduction of the gametophyte, comparable to that shown by *Salvinia* and *Azolla* (in the male prothallus), is of considerable interest, as showing how easily such reduction may be brought about in relation to external conditions. It will be important to learn whether *Todea barbara*, Moore, which is not a filmy species, would behave in the same way, and what result would be obtained by placing the sporangia of other Ferns under various conditions. It is possible that a study of this subject may yield information bearing on reduction of the gametophyte in other phyla besides the Filicineae.

Summary.

When plants of *Todea Fraseri* are kept in a sufficiently damp atmosphere, the sporangia do not dehisce, and a number of spores germinate in the closed sporangia. In drier air the sporangia dehisce normally.

[1] It would be interesting to know whether dehiscence of the sporangium is ever prevented by dampness in the natural habitats of these species.

When a considerable number of spores germinate in the sporangium, some of the prothalli (apparently not those first formed) produce antheridia. Usually a single antheridium is borne terminally on a prothallus of only three or four cells (occasionally of one or two cells), and in these specimens the rhizoid is generally not developed.

Sporangia, whether detached, or attached to a pinnule or pinna, behave similarly to those attached to the living plant. If kept sufficiently damp to prevent dehiscence (and with other conditions favourable), intrasporangial germination takes place, and prothalli bearing antheridia are ultimately formed. Antheridia were found in one experiment after twenty-one days. The prothalli do not appear to be capable of bursting the wall of the sporangium, and ultimately die. Motile spermatozoids were not seen, but once or twice the bursting of the antheridium and the emission of the sperm-cells were observed.

Free spores, placed under the same conditions as the sporangia, never produced dwarf male prothalli, but formed normal prothalli, which had not developed sexual organs at the conclusion of the experiments.

Free spores germinate in the dark in tap-water at the temperature of the experiments (roughly between 16° and 21° C.), and the prothallus may at any rate reach a six- or seven-celled stage. Spores also germinate in the closed sporangium in the dark, and under favourable conditions dwarf prothalli with antheridia are produced.

In *Todea hymenophylloides* the spores germinate less readily than in *T. Fraseri*. This may be due to the smaller amount of oil present in the spores of the former species. In *T. hymenophylloides* prothalli bearing antheridia were found in closed sporangia in only one or two experiments; they were not seen in sporangia on the living plant, though germination of a few spores was observed several times. As in *T. Fraseri*, free spores always produced normal prothalli. In *T. Moorei* and *T. superba* slight germination of spores in the sporangium was observed, but these species were not used in experiments.

A possible explanation of the formation of dwarf male prothalli in the sporangium is suggested. The mechanical hindrance to the growth of the prothalli caused by the pressure of the wall of the sporangium probably causes concentration of certain organic food substances. This may lead to special nutrition of the protoplasm, and this again may cause the precocious formation of sexual organs. Scarcity of water in the sporangium may perhaps have a similar influence.

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EXPLANATION OF PLATE XVI.

Illustrating Mr. Boodle's paper on Dwarf Male Prothalli in *Todea*.

Lettering used in several figures: *e.*, exosporium; *a.*, cavity of antheridium; *r.c.*, rhizoid-cell; *r.*, rhizoid; *s.*, sperm-cells.

Figs. 1–7. *Todea Fraseri*.

Fig. 1. Microtome-section through a closed sporangium, showing germinated spores cut in different directions; eight antheridia are visible. × 90.

Fig. 2. Section of one germinated spore from the same sporangium. The antheridium at the top contains sperm-cells, and is borne by the dwarf prothallus, the base of which is enclosed by the ruptured exosporium. × 390.

Fig. 3. Prothallus with antheridium from a closed sporangium, exposed to daylight, after 21 days; a rhizoid-cell is present. × 260.

Fig. 4. Data as in last. There is no rhizoid-cell, but the rudiment of a lateral branch (*l.*) has been formed. × 260.

Fig. 5. Prothallus (enclosed by exosporium) and antheridium from a closed sporangium; darkness, 26 days. × 260.

Fig. 6. Prothallus grown from a free spore on damp blotting-paper; darkness, 26 days. × 260.

Fig. 7. Small antheridium borne by two prothallial cells, one of which probably represents the stalk of the antheridium. From a closed sporangium; darkness, 22 days. × 260.

Fig. 8. *T. hymenophylloides.* Small prothallus with antheridium (containing chlorophyll). From a sporangium; daylight, 32 days. × 260.

Fig. 9. *T. hymenophylloides.* Prothallus from a sporangium; daylight, 32 days. × 260.

Fig. 10. *T. hymenophylloides.* Prothallus from a sporangium; daylight, 46 days. The upper cells probably represent an abortive antheridium. × 260.

Fig. 11. *T. Fraseri.* Prothallus from a free spore on damp blotting-paper; daylight, 36 days. × 260.

Fig. 12. *T. hymenophylloides.* Prothallus from a free spore on damp blotting-paper; daylight, 32 days. × 260.

Fig. 13. *T. Fraseri.* Spore germinating in tap-water; daylight, 3 days. × 260.

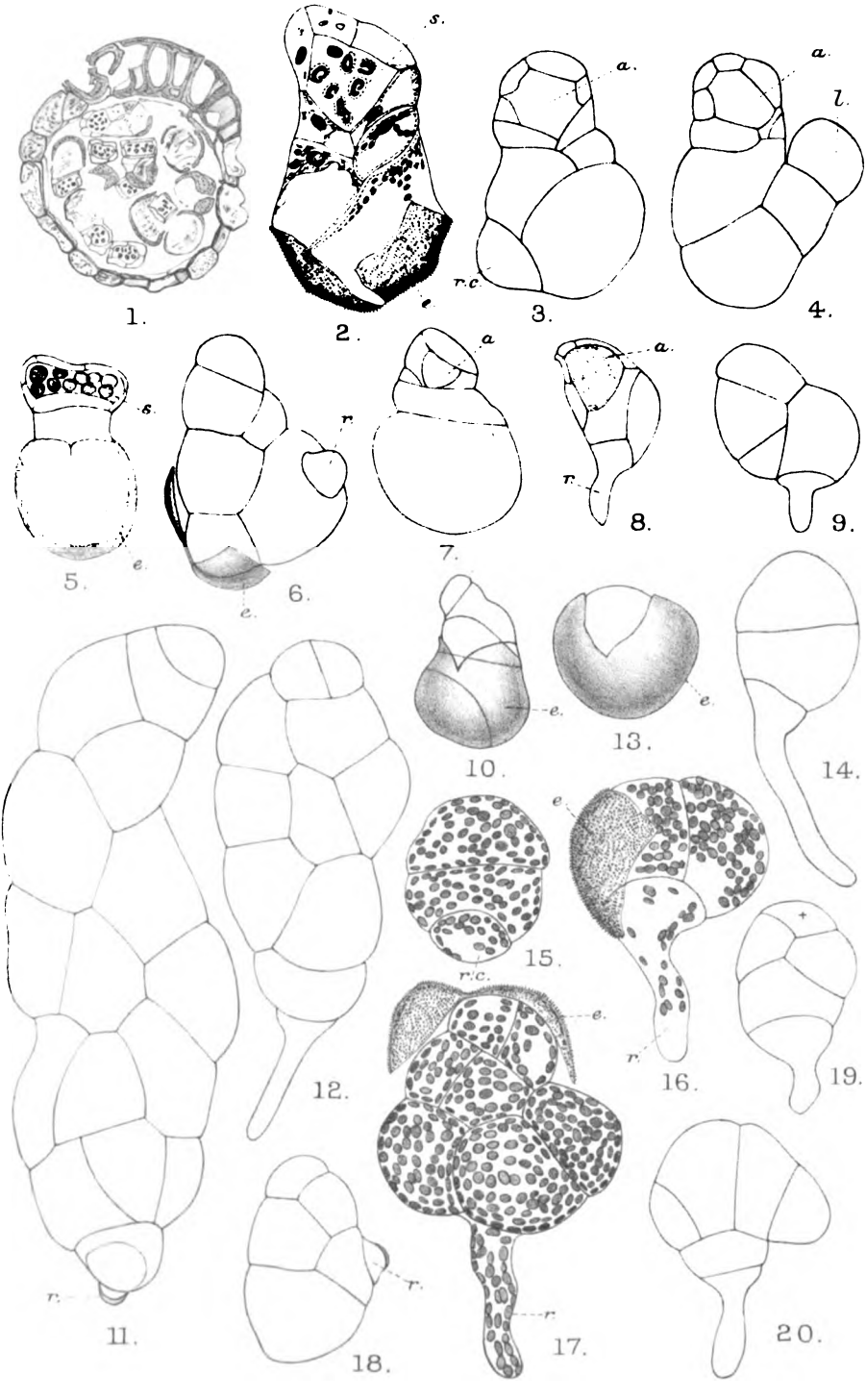
Fig. 14. *T. Fraseri.* Young prothallus from a spore in distilled water; daylight, 3 days. × 260.

Fig. 15. *T. hymenophylloides.* Young prothallus from a spore in water; darkness, 25 days. × 390.

Fig. 16. *T. hymenophylloides.* Young prothallus from a spore in a sporangium attached to a pinna on damp blotting-paper; daylight, 13 days. × 390.

Fig. 17. *T. hymenophylloides.* Prothallus from a spore in water; daylight, 19 days. × 390.

Figs. 18–20. *T. Fraseri.* Prothalli from spores on damp blotting-paper; darkness, 33 days. × 260.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The Histology of the Sieve-Tubes of Angiosperms.

BY

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With Plates XVII and XVIII and thirteen Figures in the Text.

PART I.

HISTORICAL.

NUMEROUS attempts have been made to arrive at a clear understanding of the histological structure of the sieve-tubes of Angiosperms, and of their relations to the other elements of the phloem.

Among the most important of the earlier works on this subject are the papers of Wilhelm, Russow, and Janczewski which appeared in the years 1880–3. These three investigators were concerned chiefly in examining the structure of the mature sieve-plate and its mode of formation, although Wilhelm's work in this direction occupies only a small space in his beautiful monograph on the phloem of *Vitis*.

Fischer's papers are the next in chronological order to which it is necessary to draw attention, and in them physiological rather than histological questions are considered and very little light is thrown on the origin of the sieve-plate. An interesting paper, from the point of view of sieve-tubes in general, appeared in 1887, by Oliver, which dealt with the formation of the callus in algal sieve-tubes, and reference will be made to it later.

Lecomte's elaborate thesis appeared in 1889 and is the next important paper on our subject, dealing entirely with angiospermous sieve-tubes. It is prefaced by a well-written historical summary, so that it is unnecessary to give more than a short critical account of earlier work by way of introduction to the present research.

Ten years later—in 1899—Perrot published a separate treatise, entitled 'Le Tissu criblé', the important part of which, from the point of view of this paper, is the excellent historical summary of previous research. Among the most recent attempts to solve the question of the structure and mode of origin of the angiospermous sieve-plate will be found the

[Annals of Botany, Vol. XXII. No. LXXXVI. January, 1908.]

paper by Strasburger on protoplasmic connexions, published in 1901; as this will be discussed critically in subsequent pages no account of his views need be given here. In the same year a paper on the sieve-tubes of *Pinus* appeared, by the author, which is intended to throw light not only on the phloem of Gymnosperms but also on the histology of sieve-tubes in general. This was followed by a preliminary note on the sieve-tubes of certain Angiosperms in 1903, of which the present long-delayed paper gives a more complete account.

In spite of Perrot's historical summary, it will help to a better understanding of the present position of sieve-tube research if the principal results of the various authorities are briefly stated and criticized. In particular the points of difference will be indicated in order that the nature and scope of the problems still outstanding may be clearly realized. Some of these it is hoped may have been solved by the present research.

Wilhelm worked especially with *Vitis* and to a smaller extent with *Cucurbita*, and his principal results from the histological point of view relate to the sieves and callus. With regard to the companion cells and the sieve-tubes, however, he has pointed out their origin from a common mother-cell, and he draws attention to the granular protoplasmic contents of the companion cells and to their nuclei. He noticed also the numerous corresponding pits in the cell-walls between these cells and their sieve-tubes and the almost complete absence of pits in the walls between companion and cambiform cells. The callus is considered to be formed from the cellulose of the sieve-plate, and its origin in the pits of the young sieve-plates and the subsequent perforation of these pits by the sieve-pores are described. In addition to this he shows that the development and character of the sieve-fields on the long walls of the sieve-tubes agree exactly with the sieve-plates of the cross walls. The reopening of the sieve-plate in *Vitis* after the winter rest is described with some figures, though the way in which this condition is brought about does not appear to have been properly understood.[1] The callus of old sieve-tubes which have ceased to function as such is considered by Wilhelm to serve as reserve material. The short sieve-tubes which cross the medullary rays and link up adjoining groups of sieve-tubes are also accurately described and figured, but very little information is given about the development of either sieve-plates or sieve-fields, though the early appearance of the callus was noticed.

This latter question is attacked more particularly by **Janczewski**, but his researches have not thrown very much light on the subject. It is rather to **Russow**,[2] whose work on the protoplasmic connexions was of so interesting a nature, that we are indebted for much valuable information on the structure of the sieves. Owing to the modification of some of his former

[1] Wilhelm, l. c., p. 37, Figs. 89 and 90, Pl. VII.

[2] Russow, in Sitzber. der Dorpater Nat. Ges., 1882; *Trans.* in Ann. des Sc. Nat., 1882.

views in connexion with his researches on the continuity of protoplasm,[1] and also, no doubt, to the absence of figures, his conclusions have not received the recognition which they deserve. Though his methods were of a very drastic nature it is remarkable that they have yielded results which have been proved to be substantially correct by our modern histological technique. He distinguishes clearly between the callus and the slime-strings which perforate it, not only in the large sieve-plates but also in the sieve-fields, and shows that the strings in the canals of the sieve-tubes are smooth and homogeneous throughout. He considers therefore that they cannot be of a protoplasmic nature, though in his earlier paper these strings were regarded as being of the nature of callus, owing to the brown coloration they gave with his callus reagent. In sieves which function for more than one year Russow realized the important part played by such strings (or *Stifte*) in the reopening of the sieve-plate after the winter's rest, though he does not seem to have understood either the condition of the callus-plate during the dormant period or the method by which it again becomes functional.[2]

With regard to other connexions in the phloem Russow could get no proof; he was unable to see any threads between the sieve-tubes and companion cells, or between these latter and the bast parenchyma or the medullary ray cells. No doubt his failure to obtain results in these cases was largely due to the disorganization of the cell-walls by the reagent employed.[3] His views on the early history of the sieve-plate differ from those of Wilhelm and Janczewski. These observers held the opinion that the callus arose in little spheres in pairs on either side of the young sieve-plate, owing to the transformation of the cellulose. These then fused together to form the stoppers of callus filling the pores of the sieve-plate, and finally the stoppers are pierced by the connecting filaments.

Russow, on the other hand, was of the opinion that the young sieve-plate is first pitted with corresponding pits on either side of the membrane; the callus is then formed in these little depressions by the protoplasm and not by a transformation of the cellulose.[4] The callus appears in the form of little basins, with a polygonal outline, and Russow attaches considerable importance to its shape as proof that the callus substance is formed by the contents of the sieve-tube and deposited on the surface of the sieve; the gradual overlaying of the cellulose sieve and the great size to which the callus may subsequently attain afford further proof, he considers, of the correctness of these views.

[1] Russow, l. c. 1883, p. 570.

[2] According to Janczewski, l. c., p. 145, Figs. 10–12, Pl. VIII, the sieve is entirely shut by a homogeneous callus mass in *Vitis*, and is reopened with the formation of striae about mid-April.

[3] Russow used about 81 per cent. sulphuric acid.

[4] Gardiner, 'On the constitution of the cell-wall and middle lamella', in Proc. Camb. Phil. Soc., vol. v, p. 15, ibid., 'Observations on the constitution of callus', p. 230, agrees with the views put forward by Russow as to the protoplasmic derivation of the callus, and considers this substance to be of the nature of a starchy mucilage.

Neither the solution of the cellulose lamella between the two opposed callus-basins nor the formation of the connecting filaments was observed.

Both Janczewski and Russow have investigated the sieve-tubes of Gymnosperms also, but their results need not detain us here, since they have been discussed elsewhere.[1] The papers of **Fischer**,[2] which follow in chronological order, deal with the physiological rather than with the histological aspect of our subject, one of his objects being to show that the ordinary contracted appearance of the sieve-tube contents and the characteristic masses of slime (*Schleimkopf*) seen on one side of the sieve-plate have been artificially produced, and that in the normal condition the sieve-tube contents are homogeneous.

One of his papers is of some histological interest, since he employed Russow's method, and two of his figures probably show the sort of results which Russow must have obtained. In both cases the results are artificial and untrue, the cell-walls being enormously swollen. In the one case the wall between two sieve-tubes shows stratification with 1–4 threads in each pit, and, in the other, direct continuity by single hair threads[3] is figured between a companion cell and a sieve-tube.

In the summary of his results he states that the sieve-tubes are in 'direct continuity' with one another through fine threads and with the companion cells,[4] and that the cambiform cells are in continuity with one another, but not with either the sieve-tubes or the companion cells. Fischer's results seen by the light of his figures are so misleading that they cannot be considered as a very valuable contribution to the histology of the phloem tissues. The next work on this subject is **Lecomte's** careful study of the phloem of Angiosperms[5], a good deal of which is occupied with questions of histology. His own work is collected into nine chapters, of which his chapters IV, on the Development of the Sieves,[6] and V, on the Development, Form, Structure, and principal reactions of the Callus,[7] concern us more particularly in this paper.

Lecomte, after a useful review of previous work, proceeds to give the results of his own researches, which in some points differ fundamentally from those to which allusion has already been made. According to him,[8] the young thin membrane—the future sieve-plate— is at first composed of some nitrogenous body; this soon becomes coated with cellulose in such a way that the cellulose forms intersecting bands, leaving the meshes of the

[1] Hill, A. W., Histology of the sieve-tubes in *Pinus*, in Ann. Bot., 1901, vol. xv, pp. 576–80.

[2] Fischer, *v.* list of Literature cited at end.

[3] There is no doubt that the so-called threads are the pit fillings of paired pits so drawn out by the intense swelling of the wall that they appear like a fine continuous thread.

[4] Fischer, A., Neue Beiträge zur Kenntniss der Siebröhren. Ber. über Verhandl. der kön. Sächs. Ges. d. Wissen. zu Leipzig, 1886, p. 297.

[5] Lecomte, in Ann. des Sc. Nat., Bot., Sér. vii, 1889, vol. x, p. 193.

[6] Ibid., p. 244.

[7] Ibid., p. 258.

[8] Ibid., pp. 248 and 250, Fig. 9, Pl. XXI, Fig. 12, Pl. XXII.

original substance which will become the pits of the young sieve-plate; the meshwork thus tends to be of greater thickness than the rest of the plate. After this the substance occupying the meshes swells, forming projections on either side of the membrane, and stains with aniline blue, so that the contents of two tubes appear to be united by blue rods which occupy the axes of the meshes.[1] Gradually the whole of the substance filling the mesh takes on this blue coloration. Osmotic exchanges take place at first through the substance filling the meshes of the sieve-plate, but later communication between two adjoining tubes is effected by a definite mucilaginous filament traversing a canal or hole in the plate owing to the replacement of the original substance of the meshes by the invading filaments of the albuminous contents of the sieve-tubes.

The parietal protoplasm also accompanies these filaments, so that there is communication not only of the slime but also of the protoplasm of the sieve-tubes. The exact way in which the change from the embryonic to the mature mode of communication takes place is by no means clear from Lecomte's account. The development may not always go as far as this; in Gymnosperms, for instance, and probably in many Angiosperms also, the meshes, according to his view, may always be occupied by the primitive substance of the membrane and may only afford places more suitable for osmotic exchange than other parts of the sieve-plate.

Before criticizing these views of Lecomte on the development of the sieve-plate, it will be more convenient to give a brief résumé of his opinions on the formation of the callus. He criticizes Russow's results,[2] with which he entirely disagrees, and comes to the conclusion that the callus forming the callus-pads is due to the swelling of the thin layer of the substance which covers the cellulose network of the sieve-plate,[3] but that the cellulose network itself never gives rise to any callus. The first callus layer is an integral part of the membrane of the sieve-plate, and then this layer thickens at the expense of the albuminous contents of the tube.

Thus this substance which fills the meshes of the young sieve-plate and also covers over the cellulose framework functions first of all—and in some cases always[4]—as the pathway for osmotic exchanges. The portions which are not transformed into mucous strings—i. e., the parts covering the cellulose network—then swell up to join the first layer of callus.

According to Perrot's account of the work of Lecomte and Léger,[5] the

[1] Perrot, 'Le Tissu criblé', p. 38, and Fig. 19.

[2] Lecomte, l. c., p. 263. [3] Ibid., p. 261.

[4] Ibid., p. 260. After the penetration of the meshes, the substance covering the cellulose network shrinks to an almost imperceptible layer, since communication is now direct, but in those Dicotyledons where he was unable to show the perforations he found the wall notably thicker than all the others. This is due to the fact that the osmotic changes take place exclusively here, and the non-cellulose portion retains the first stage of swelling seen in the young sieve-plates.

[5] Cf. Perrot, l. c., p. 36; cf. Lecomte, pp. 248 and 260. It does not appear that Lecomte realized that the substance of his meshes which stains blue with aniline blue is really callus (*v.* Conclusion,

substance of the meshes must thus be transformed first into callus and afterwards progressively, in certain cases, into an albuminous substance.

Lecomte's results, which seem to have been generally accepted (certainly by French botanists), not only make the early history of the sieve-plate more complicated than really appears to be the case, but also include several erroneous conceptions as to the state of the active sieve-tubes in Gymnosperms and some Angiosperms. There seems every reason to suppose that the sieve-tubes in these two groups of plants are—at any rate in the active condition—always in communication with one another by means of mucilaginous slime-strings.[1] Further, the callus is always visible before any perforation of the membrane by the slime-string has taken place, as Russow and Wilhelm observed,[2] and Lecomte does not appear to have noticed the little paired basin-shaped patches of callus separated from each other by a thin intervening piece of the membrane, which were accurately described by Russow and form so marked a feature of the developing sieve-plate. Nor does he allude to the callus lining the holes of the sieve-plate, which has been well figured by Wilhelm.[3] According to Lecomte the callus first arises as a transformation of the substance covering the cellulose network of the sieve, whilst according to Wilhelm, Janczewski, and Russow, with whom on this point the present researches are in accord, the callus makes its appearance in the meshes, depressions, or pits of the young sieve-plate, i. e., the spaces between Lecomte's hypothetical intersecting bands of cellulose. His theory of the origin of the perforations in the sieve-plate and of the value of the substance of the meshes as the pathway for diffusion will be referred to again later. It is enough to add here that the knowledge gained by recent histological work on the cell-walls and protoplasmic connexions makes this theory highly improbable. There are other points of which the explanations are by no means clear, such as the nature and function of the striae in old sieves and the way in which sieves may be reopened after the winter's rest. In concluding this criticism of Lecomte's work it must be remarked that his figures are not sufficiently clear in many cases to enable one fully to understand his meaning.[4] The question of 'connecting-threads' in the phloem tissues does not appear to have engaged his attention.

A contribution to this subject of the sieve-plate was made by **Léger**,[5]

No. 16, p. 320). 'Le cal est dû au développement exagéré de la mince couche de la membrane qui recouvre les filaments de cellulose.'

[1] Hill, Ann. Bot., vol. xv, p. 590, &c.

[2] Wilhelm, Figs. 12, 14, 15, Pl. II; Fig. 25, Pl. III; Figs. 114, &c., Pl. IX.

[3] Ibid.

[4] e. g., his figures of sieve-plates in section and surface view, Figs. 9 and 10, Pl. XXI, and Figs. 12, 21, and 22, Pl. XXII, do not correspond in an intelligible manner, and they are even less clear when copied by Perrot, l. c., p. 37, and the sieve-plates of Figs. 5 and 6, Pl. XXI, and Figs. 14 and 17, Pl. XXII, do not appear to represent real structure.

[5] Léger, Mém. de la Soc. Linn. de Normandie, tome xix, 1897, p. 68.

who finds that it is pectic in composition, when deprived of its callus, and gives no cellulose reactions.

Such then was the condition of our knowledge of the sieve-tube and phloem histology in 1899 when **Perrot** wrote his memoir, in which he accepts nearly all Lecomte's results. The next contributions of a serious character are those of **Strasburger.** In his important paper on protoplasmic connexions,[1] published in 1901, he still held the view put forward in an earlier work[2] that with the formation of the callus-rod from the original connecting-threads the complete development of the sieve-plate has been reached. In his review[3] of my paper on the sieve-tubes of *Pinus*,[4] he accepts certain of the conclusions there put forward and agrees that it is the slime-string and not the callus-rod which plays the important rôle in the communication between two sieve-tubes.[5]

In the paper published in 1901 he also traces the history of the development of the sieve-plate and sieve-fields in *Wistaria chinensis* (*Kraunhia*), and other Angiosperms, and, since the results there stated differ considerably from those in general acceptance at that date, it will be necessary to give some account of them and also to add some criticisms.

According to Strasburger, the young sieve-plate membrane soon after its formation becomes covered by a network due to local thickening. The whole membrane is then overlaid by a substance which is characterized by the readiness with which it stains blue with aniline blue, and at this period the fine protoplasmic threads are seen in the sieve-fields.[6] The threads, however, soon begin to take up aniline blue, and, in favourable cases, a surface view effect can be obtained of a fine network in a sieve-field corresponding to this perforation of the membrane (*v.* Fig. 30, Pl. XIV). So far this agrees with the course of development given by him for *Pinus*, to which reference has already been made. The further stages of the development, however, are different. The aniline blue staining substance which overlies the whole sieve-plate accentuates particularly the height of the network, and continuity across the unstained portion of the membrane is

[1] Strasburger, in Jahrb. f. wiss. Bot., Bd. xxxvi.

[2] Strasburger, Leitungsbahnen, 1891, pp. 60 et seq.; *v.* Hill, Histology of Sieve-tubes of *Pinus*, Ann. Bot., vol. xv, 1901, pp. 582–3.

[3] Strasburger, in Bot. Zeit., vol. lx, 1902, p. 49.

[4] Hill, Ann. Bot., vol. xv, p. 575.

[5] l. c., p. 47, Strasburger states that in his 'Kleines Practicum,' fourth edition, he had corrected the figure of the sieve-plate of *Pinus* from the figure given in his paper before my work was published; his figures are certainly somewhat altered, but in both cases they appear to be so badly reproduced as to be almost unintelligible. Moreover, in the 'Practicum,' fourth edition, there is no adequate explanation of the altered figures in the text; cf. p. 85, Fig. 49.

[6] Cf. Strasburger, l. c., Fig. 29, Pl. XIV. The sieve-fields in Strasburger's sense are the small sieve-areas or pits of the single sieve-plate, they thus correspond to Lecomte's meshes of the sieve-plate network. Other writers limit the term 'sieve-fields' to the thread groups on the *lateral walls* of the sieve-tubes; and it is so used in this present paper.

effected by stained callus-threads; a surface view may sometimes be seen showing separate blue dots which are his callus-threads, and Figs. 31 and 32, Pl. XIV, illustrate this stage in the development.[1] The network or framework of the sieve-plate continues to project still more above the general surface of the membrane owing to the progressive conversion of its substance into callus, and at the same time the perforation of the several sieve-fields takes place. The mucous contents of the sieve-tubes begin to bore through the centre of the membrane of each field from one side only; this boring is not due to the fusion of the callus-threads which traverse the membranes of the sieve-fields, for it commences with a small hole in the centre of the field, and this gradually increases in diameter until the whole of the field area is occupied by a slime-string. By means of these strings the slime masses of adjoining sieve-tubes are in communication (cf. Figs. 33–35, Pl. XIV). The conversion of the thickened portion, or network, of the sieve-plate into callus still continues, and there results eventually a narrowing of the several pores (Figs. 36–37, Pl. XIV).

It may be noted from the figures (Fig. 36, Pl. XIV; Figs. 38 and 39, Pl. XV) that during the progress of this narrowing the callus has increased in size, whilst the cellulose or substance of the original membrane has decreased. Finally the thickening callus masses of the framework fuse together and the shutting of the sieve-plate by a callus-rod is complete (Fig. 40, Pl. XV). In a longitudinal section of the callus-mass the remains of the original membrane can still be seen as a series of nodes, and in a surface view, on the solution of the callus, this same primary membrane is left behind as an open network (Fig. 42).[2]

The finer network seen in *Pinus*, representing the remains of the sieve-field membrane (cf. Figs. 26 and 27), is therefore absent in *Wistaria*, and the network of the latter and of Angiosperms generally is considered to be homologous with that larger network which encloses the sieve-fields in *Pinus*.

Strasburger regards the callus in *Pinus* as derived from the swellings of the ends of the changed protoplasmic threads, whilst in the sieve-plates of *Wistaria* and Angiosperms he considers that callus is formed by the transformation of the substances of the main network of the cell membrane. He thus agrees, in the main, with Lecomte as to the mode of origin of the callus-pads in Angiosperms, and alters the opinion previously advanced in the 'Leitungsbahnen', that the callus formation in Angiosperms and Gymnosperms must follow the same line of development. That a substance, so constant in its occurrence as the callus, and found in such diverse groups

[1] It seems necessary to point out here that, although his Figs. 29–32 are all drawn to the same magnification, the surface views Figs. 30 and 32 do not appear to correspond with their respective sections, Figs. 29 and 31. It is also difficult to understand Fig. 30, and in Fig. 31 the callus-rods, into which the protoplasmic threads are supposed to change, are not indicated.

[2] Fig. 42, Pl. XV, shows such an empty plate in surface view: but it is not easy to understand its correspondence with the section in Fig. 41.

of plants as the Brown Algae, Gymnosperms and Angiosperms, always in the same connexion with the perforations of the sieve-plates of the sieve-tubes, should have a different origin in the different groups, seems to be unlikely. My own results suggest that the mode of formation of the callus in connexion with the sieve-tubes takes place in the same way in the different groups of plants.[1] With regard to the smaller sieve-pits on the lateral walls of the sieve-tubes—the sieve-fields of Wilhelm[2] and others—Strasburger[3] considers that they differ but little from the pits in the walls of ordinary parenchymatous cells, and do not therefore agree in structure with the terminal sieve-plates. They differ from ordinary pits, however, since their threads are said to be transformed into callus, whose ends then swell—as he has described in the Conifers—to form callus-heads and eventually fuse together to form the lateral callus-pads (*v.* Fig. 44, Pl. XV).

Thus it appears that in the same sieve-tube the callus-pads of the terminal and of the side walls have a different mode of formation. On the disappearance of the callus by solution there is no solution of the membrane separating the several callus-threads as was described in *Pinus*; the membrane thus retains its original thickness and is perforated by empty canals (*v.* Fig. 45, Pl. XV). In this respect, therefore, these lateral pits of Angiosperms differ from those of the Conifers in which, according to Strasburger,[4] a fine network is seen in the sieve-fields left naked by the solution of the callus; for only the thickening layers have been dissolved and the middle lamella is left behind. The nodules therefore which he sees and figures in the middle of the sieves of young sieve-tubes are not swellings on the callus-threads, but represent the thicker parts of the middle lamella forming the fine meshwork. As this question has already been discussed in a former paper[5] it is unnecessary to go into details again here. Suffice it to say that the condition of the lateral thread groups of Angiosperms, after the solution of the callus, appears to be essentially similar to the sieve-areas of Conifers. In both cases the membrane appears to be perforated by empty canals and the refractive nodules of the Conifers have vanished. Nothing[6] has been found to necessitate in any way an alteration of the views already put forward which tend to unite the

[1] Cf. the paper by Miss Sykes in this present number of the Annals.

[2] Wilhelm, l. c., Figs. 34 and 35, Pl. IV.

[3] Strasburger, l. c., p. 531, Figs. 43-45, Pl. XV.

[4] Ibid., p. 525, Figs. 26 and 27, Pl. XIV.

[5] Cf. Hill, l. c., pp. 584, 592, Fig. 13, Pl. XXXI.

[6] In the review of the paper on the sieve-tubes of *Pinus*, Strasburger, in Bot. Zeit., vol. lx, p. 57, 1902, adheres firmly to his views about the median nodules of the sieve-plates. Material has been examined which has been fixed in various ways, including alcohol material, and no trace can be seen of the conspicuous highly refractive nodes situated at the middle lamella between the paired callus-rods, after the callus has disappeared; cf. Hill, l. c., pp. 587-8, Figs. 11 and 13, Pl. XXXI; and Fig. 21, Pl. XXXIII. In the explanation to Fig. 11, Pl. XXXI, p. 608, for nodules (*mn*) read (*m*), and for median nodes (*m*) read (*mn*).

phenomena attendant upon the development of the various types of sieve-tube connexions throughout the vegetable kingdom into one harmonious series of events.

It may be noted here that Strasburger says nothing further as to the character of the sieves in Conifers.[1] In the fourth edition of the 'Kleines Botanisches Practicum' the figures of the sieves of *Pinus*[2] have, as I have already stated, been altered, and the callus is represented as being pierced by fine threads, but whether he considers that the method of callus formation put forward in 1901 still holds, or whether the results of the paper on the histology of *Pinus* have altered his opinions, is not clear. With regard to the sieve-plate of Angiosperms the statement is made[3] that in consequence of my results with *Pinus*[4] a correction is necessary in the sense that slime-strings and not callus-rods are formed from the 'Plasmodesma' or connecting-threads. How the acceptance of these results affects his views on the boring of the fields of the sieve-plate by one large central slime-string, when according to his new point of view the young field should be occupied by a group of slime-strings,[5] or how the callus which lines the pores of the sieve-plates is formed, is not stated. In the same way the character of the lateral thread groups is left in an unsatisfactory condition, for no explanation is given of the relation of the slime-strings to the callus, which was supposed to be formed by the swelling up of the free ends of the transformed protoplasmic threads[6] as in Conifers.

Two other papers require a brief notice before closing this résumé. **Kuhla,**[7] in his paper on the protoplasmic connexions in *Viscum album*, published in 1900, includes some account of the sieve-tubes of *Cucurbita* and of *Viscum*. He worked with alcohol material of *Cucurbita* fixed by boiling, according to Fischer's method[8], and stained by Meyer's Pyoktanin method.[9] The points which he wished to settle are clearly stated in his paper,[10] but, though figures are given which purport to answer the questions propounded, there is reason for believing that the appearances figured do not, in most cases, really correspond to actual structures.

In the first place the callus is neither mentioned in the text nor referred to in the figures, which therefore renders Figs. 8, 17, and 35,

[1] Strasburger, Bot. Zeit., lx, pp. 49–53.

[2] Bot. Pract., ed. iv, 1902, p. 86, Fig. 49 *a* and *b*, p. 85. In these two figures fine threads each with a dark median node are shown. In Fig. *b* there seems to be a suggestion of a median nodule enclosing the nodes, which does not appear in *a*. The figures, however, are so vague and indefinite, and there is so little allusion to them in the text, or to the fact that they differ from those previously published, that it is difficult to be certain about them. There is no proper explanation of Fig. 49 *c*, and it does not apparently harmonize with either *a* or *b*.

[3] Strasburger, B. Z., l. c., p. 52.

[4] Hill, Ann. Bot., vol. xv, 1901.

[5] Cf. Strasburger, Pringsh. Jahrb., 1901, Fig. 32, Pl. XIV. in the light of B. Z., 1902, p. 52, § 2.

[6] Strasburger, 1901, pp. 531, 532.

[7] Kuhla, F., Bot. Zeit., 1900, p. 29.

[8] Fischer, 1886, Beit. zur Kennt. der Siebröhren, Leipzig.

[9] The *Viscum* material appears to have been fixed in 1 per cent. Osmic acid.

[10] Kuhla, l. c., p. 39.

Pl. III, inaccurate. His figure (Fig. 10) of the threads between a sieve-tube and a cambiform cell in *Cucurbita* is also inaccurate for the same reason, since callus is always present in the sieve-tube side of these pit-closing membranes. Connexions between the sieve-tubes and the companion cells of *Cucurbita* are figured in Figs. 7 and 9, but these two figures are very different in character, and Fig. 7 corresponds very closely with his Fig. 8, which represents a sieve-plate[1]. It seems possible, therefore, that these thick threads may really be the stained pit fillings in the narrow paired pits of the companion cell-wall which have become narrowed by the swelling of the wall owing to the nature of the treatment to which the tissues have been subjected.[2] Even if the threads in Fig. 9 are real threads, the swollen state of the wall has produced a highly artificial effect. With regard to the presence of threads between the other tissues of the phloem, there is no doubt that they exist; Kuhla's figures of these are, however, unsatisfactory, and this is largely due to the great swelling of the wall and to the unsatisfactory and misleading nature of Meyer's method as a method for research.[3]

Fig. 35 appears to have no adequate reference in the text[4]; although it purports to be a very young sieve-plate, it is, I think, without doubt an old and empty plate with the sides of the pores stained by Pyoktanin.

Kuhla's results with the sieve-tubes of *Viscum* are worthy of more serious consideration and will be referred to again later, but it is as well here to point out certain errors of observation. In the first place the end-wall or sieve-plate of *Viscum*[5] is provided with callus, like that of the sieve-plates of other plants, and is pierced by slime-strings. Also, owing to the swelling of the walls, the very numerous pits in the side walls of the sieve-tubes have been entirely overlooked, and the threads are drawn with a regularity foreign to their normal distribution.[6] Nevertheless it should in all fairness be stated that the long walls between the sieve-tubes and the companion cells and between two adjoining sieve-tubes are filled with threads somewhat after the manner he has indicated.

A few remarks about sieve-tubes are made by **Kienitz Gerloff**[7] in his recent paper, and a figure of a sieve-plate of *Gagea lutea* is given, but no callus is indicated, and there is nothing further on this subject in his paper which need claim our attention.

It is possible that certain general statements made in the preliminary

[1] Cf. Kuhla, Bot. Zeit., 1900, p. 40.

[2] Ibid., p. 40; Eau de Javelle and Chlor-Zinc-Iod. appear to have also been used on the same sections; cf. Fischer's results, *v.* p. 248.

[3] For the above reasons and from my own results with *Viscum* obtained by other methods I am inclined to think that Kuhla's elaborate numerical calculations may not be thoroughly reliable.

[4] Perhaps p. 41 at the foot refers to this Figure. [5] Cf. Kuhla, l. c., Fig. 17.

[6] Kuhla, l. c., pp. 42 and 43, Figs. 12–14; cf. Hill, Fig. 26, Pl. XVII.

[7] Kienitz Gerloff, Ber. der Deut. Bot. Ges., xx, 1902, p. 108.

account[1] of the present research with reference to the structure of the young sieve-fields may require modification, but all such questions will be referred to in the course of this paper.

Summary:—From the review of the literature presented in the previous pages it will be seen that more than one account of the developmental history of the sieve-plate and other questions of phloem histology has been given during the last thirty years. Wilhelm's work, as far as it goes, agrees with the results of the present research, and had he been in command of a better technique there can be little doubt that he would have been able to work out the finer histological details. Nearly all his work can be accepted as it stands, and serves as the foundation on which the complete history may be built up.

The importance of Russow's work, taken in conjunction with Wilhelm's results, lies in the fact that the relation of the slime-strings to the callus-rods both in adult sieve-plates and in sieve-fields (lateral pits) is definitely established. Russow also contributed to the early history of the sieve-plate, and his description of the first appearance of the callus is apparently in exact agreement with the results of the present research.

The opposed views of the formation and development of the callus by these two botanists are probably both partly true, for, as will be shown later, it seems likely that the earliest formation of the callus is due to a local transformation of the cell-wall, whilst the activity of the protoplasm is responsible for its subsequent increase.

Lecomte, influenced no doubt by Mangin's work on the cell-wall, produced a totally different theory of the origin of the sieve-plate of a complicated character, since the young end-wall is considered as being composed of two different substances forming a meshwork and interstitial matter respectively. The latter substance is considered in some cases to be gradually transformed into slime-strings, whilst in other plants there is said to be no open communication. Lecomte's views on callus formation are not very clear, but he agrees rather with Wilhelm in supposing that it is due to a change of part of the pit-closing membrane. On the whole Lecomte's work, from the histological point of view, does not advance our knowledge of the sieve-tubes to any very valuable extent, nor has he thrown any light on the presence of connecting-threads in the tissues of the phloem.

To Kuhla and Fischer we owe some knowledge about protoplasmic connexions between sieve-tubes and other phloem tissues, &c., but, beyond the fact that they have demonstrated the existence of such threads, their work cannot be considered as very trustworthy, owing to the nature of the methods which they employed.

Strasburger has attempted to carry on the investigation of the histology of the sieve-tubes from the point where it was left by Wilhelm

[1] Hill, Ann. Bot., vol. xvii, 1903, pp. 265–7.

and Russow, and has thrown light on the early history of the sieve-plate, but owing to his confusion of slime-strings and callus-rods, which is not thoroughly cleared up by his review of 1902,[1] his results are shorn of some of their value.

That he is in error about the two different methods of callus formation, whether found on the end or on the lateral walls of the sieve-tubes, will, it is hoped, be proved in the course of this paper. He also has had nothing to say about the possibility of the existence of connecting-threads between sieve-tubes and other tissues of the phloem.

Such then is the state of our knowledge of the histological details of the sieve-tubes and phloem of Angiosperms at the present time. It is to the general elucidation of the development and character of connecting-threads and subsidiary structures in these tissues that the present research—commenced in the spring of 1901—has been directed. A short preliminary note has already appeared, but owing to a journey abroad the completion of the work has been considerably delayed. Even now there are many points which must be subjected to future research, but it has seemed advisable to publish the results which have been obtained and to give a general account of phloem histology without waiting for the solution of certain small outstanding difficulties.

PART II.

The Present Research.

A short preliminary note on the present research was published in the Annals of Botany[2] in 1903, and some account of the results was given at the Southport meeting of the British Association[3] in the same year. The research has been carried out mainly on material of *Vitis vinifera* and *Wistaria chinensis*, whilst *Cucurbita pepo*, *Tilia vulgaris*, *Viscum album*, and *Phaseolus vulgaris* and *multiflorus* have also been studied in greater or less detail.

As in the case of *Pinus*, it was found that the nature of the material employed was a matter of extreme importance for the study of the development of the sieve-tubes, and it is advisable that the pieces of tissue for preservation shall be taken only from the main stems of strong and vigorous plants. The young shoots of woody plants in their first or second year of growth are seldom favourable. In order to obtain the best results it is necessary to cut a piece of the phloem out of a fairly old stem, and care must be exercised in order to insure that the cambium is removed at the same time. In *Vitis*, *Wistaria*, and *Tilia* the pieces of tissue so removed will

[1] Strasburger, Bot. Zeit., vol. lx, 1902, p. 50. [2] Hill, Ann. Bot., vol. xvii, 1903, p. 265.
[3] Brit. Assn. Report, Southport, 1903, p. 854.

show a broad band of phloem with sieve-tubes in all stages of development, from their earliest formation to their disused and collapsed condition.

In material of this kind the sieve-tubes are larger and have thicker walls than those of the young shoots; moreover, the developmental changes appear to proceed more slowly, and are more easily observed in tissues gouged out of the main stems.

The preservation of the material is probably the most important matter in connexion with this kind of histological research, and the sieve-tubes of Angiosperms appear to offer much greater difficulties than those of the Gymnosperms. This seems to be due mainly to the character of the sieve-plate, which, owing to the size of its pores, allows a shrinkage of the sieve-tube contents to take place on cutting into a stem, and produces the characteristically contracted appearance which is nearly always seen in fixed sieve-tubes. The same kind of difficulty is experienced with developing sieve-tubes, since their watery contents are enclosed in only a thin protoplasmic sac; on fixation plasmolysis readily takes place, and the contraction of the protoplasm is liable to distort the true structure of the sieve-plate with its developing slime-strings.

The artificial appearance of the fixed sieve-tube was pointed out by Fischer,[1] but his method of fixation by boiling tends to produce effects which do not represent faithfully the living structure.

Certain experiments, which have been carried out on living plants of *Cucurbita* with the intention of fixing the sieve-tubes in an uninjured condition, with the usual reagents, were to a certain extent successful, for they show a more or less uniform distribution of the slimy contents within the protoplasmic sac, and in this respect show some agreement with Fischer's results.

Methods.

The methods employed in this investigation were based on those published by Gardiner[2] in 1898. They were on the whole similar to those employed in the examination of *Pinus*, but certain modifications in detail have been introduced as occasion has required.

The material was killed either in very small pieces, consisting of cambium and phloem only, or in the form of sections cut from fresh frozen material.

All killing agents and fixatives were in aqueous solution, and comprised solutions of Iodine in Iodides, Picric acid, and mixtures of such solutions with other reagents. Solutions containing corrosive sublimate and Chromic, Acetic or Osmic acids were not found useful as killing agents.

It has been found necessary, as in the demonstration of ordinary connecting-threads, to induce a certain amount of swelling of the killed tissues in order to obtain satisfactory staining reactions, and for this reason

[1] Cf. p. 248.

[2] Gardiner, Proc. Camb. Phil. Soc., 1898, p. 508.

it is as well to omit Osmic acid from the killing solutions. The requisite amount of swelling, which often need only be very slight, can be brought about by a solution of Iodine in an Iodide, or by Picric acid or by Picro-Sulphuric acid, for two or three days or longer as circumstances may require, and the material may be swollen either in bulk or after it has been cut into sections.

The material may be preserved in Thymol water either before or after the swelling of the tissues has been effected, provided the killing, fixing, and swelling reagents have been thoroughly washed out. It is also important that the material in Thymol water should be kept in glass or india-rubber stoppered bottles, as, with ordinary corks, moulds are very liable to appear in the bottles.

Staining.

The same principles underlie the methods employed in staining as have already been published in brief by Gardiner. Certain modifications have been introduced to suit special cases, and some new methods have been devised, without, however, departing from the general principle which appears to govern the method as a whole.

Staining is effected in all cases after previous mordanting of the protoplasm. If Safranin is to be the stain, then the mordant may be a salt of Uranium or Platinum, Potassium permanganate, and also to a certain extent Iodine. Uranium and Platinum salts are usually used in conjunction with Osmic acid according to a modified formula of either Kolossow or Hermann. These latter mordants require careful washing out before the staining can take place, and they should be used for pieces of tissue rather than for sections. To overcome this difficulty, and also to shorten the time taken over the mordanting process, a modification has been introduced which is found to be of considerable value in many cases. Since both Iodine in Potassium Iodide and Uranium Nitrate are mordants for protoplasm, and the Iodine has rapid penetrating powers, it seemed likely that Uranium Iodide, should such a compound exist, would be a very useful reagent. Dr. Fenton suggested that if equivalent quantities of solutions of Uranium Nitrate and Potassium Iodide were mixed together, when required for use, the desired compound might be formed which would mordant the protoplasm of the sections placed therein. This has been tried with quite successful results; it is especially useful for dealing with sections, and, should the staining be not sufficiently heavy at the first attempt, it is quite easy to repeat the whole process one or more times.

The Safranin method of staining is the most useful for sieve-tube research, since it is possible to stain the callus with Water Blue without interfering with the stained protoplasm, and it is very important for a right understanding of the structure of the sieve-plate that staining of both

callus and protoplasm shall be possible in the same section. With the Safranin method it is possible, if deeper staining is required, either to remordant with any of the proper mordants and restain with Safranin or with Gentian Violet, or else to use the Acid Blue method after the first staining with Safranin.

The Acid Blue method, which is sometimes a useful alternative to the Safranin staining, may be used on sections cut from fixed or fresh material, but cannot be used directly with any success on material which has been mordanted for Safranin; intensification can be brought about by repetition of the process. By this method results are somewhat artificial; it is not always easy to be certain whether the effects produced are due to precipitation between the Iodine and the dye taking place in empty spaces, or whether they represent the staining of actual structure. In addition to this it is not possible to stain the callus properly with Water Blue or other reagents, since the action of the acid distorts the callus, and the action of the Water Blue tends to upset the protoplasmic staining. The unchecked use of this method has led Kuhla [1] into error with regard to the structure of the sieve-plates, &c. of *Cucurbita*, and though the modification of Meyer's Acid Violet (Pyoktanin) method, by which staining takes place in watch-glasses and not on the slide, gives fairly reliable results, it can only be used usefully in comparison with the Safranin method.

It may be as well to remark here that the success of the modification of the Acid-Violet method is due to the employment of Benzyl Blue [2], a peculiar impure dye made some twenty-five years ago by the Actien-Gesellschaft, &c. of Berlin. A dye is still sold under the same name, but it is now made by a different process, with the result that it is practically useless for the method.

A third method which has recently been devised is, in principle, like the Safranin method, over which, in some cases, it has certain advantages. Sections of fixed material may be mordanted with a weak solution of Iron Alum, and after a brief washing staining is effected by an Aniline water solution of Water Blue made up after the manner of the Safranin solution. By this method the cell-walls are not stained at all, which is an advantage over the Safranin method, and the Water Blue in this case does not stain the callus. Intensification of the staining is possible by repetition of the process, and a very dark staining of protoplasm and threads is obtained with scarcely any coloration of the cell-wall, and without any precipitation effects.

The stained sections are mounted for observation either in Glycerine with Iodine, or a mixture of Glycerine and Zinc Chloride with Iodine, or in Glycerine Jelly, and there is nothing to add to what has been already published. Preparations so mounted are found to be quite clear at the end of

[1] Kuhla, Bot. Zeit., 1900, pp. 38 et seq. [2] The value of this dye was discovered by Gardiner.

four or five years, and if remounted in a medium containing Iodine they will regain their former sharpness of detail. As in the case of *Pinus*, the material has been studied as much as possible by the use of transverse and radial sections. By this means the various stages in the development of the sieve-plate can be more readily followed, and mistakes can be avoided such as may easily be made when examining tangential sections of unknown age by the Acid Blue method.

The Active Sieve-Tube.

So much has been written about the structure of sieve-tubes, with so many conflicting details, that it may help to make what is to follow more clear if a short account of the structure of the active sieve-tube is given by way of preface.

The active sieve-tube may be regarded from two points of view, namely, with reference to the structure of the walls of the tube, and also as to the nature of the contents. Without going into details it may be stated that the contents consist of a hollow sac-like protoplasmic body in intimate communication with the protoplasmic bodies of neighbouring sieve-tubes, in the vertical direction by means of the large pores in the sieve-plates, and laterally by means of the finer connecting-threads or strings, and occasionally also by lateral sieve-plates. The protoplasmic body encloses the peculiar slimy contents, consisting of metabolized material in a state of flux, which give the sieve-tubes their characteristic appearance in fixed material. The slimy contents pass through the pores of the sieve-plate from one sieve-tube to the next—the slime-strings—but they do not come into contact with the cell-wall, owing to their enclosure in protoplasmic tubules which pass with them through the holes in the sieve-plate. A similar structure, but on a much smaller scale, appears to be shown by the groups of lateral connexions.

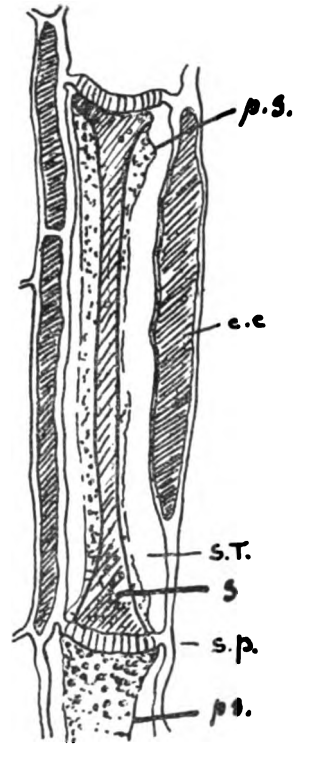

Fig. 1. A young sieve-tube (*s.t.*) in longitudinal section, showing the sieve-plates (*s. p.*) crossed by slime-strings. The tube shows the slimy contents (*s.*) within the protoplasmic sac (*p. s.*). On one side a companion cell (*c. c.*) is seen. (Copied from Wilhelm, Pl. II, Fig. 13, *Vitis vinifera*.)

The cell-walls of the sieve-tubes—at least in *Vitis* and *Wistaria*—do not appear to be sharply differentiated in their chemical composition from other cell-walls of the phloem, though according to their staining reactions

they seem to possess a somewhat higher percentage of cellulose. The sieve-plates, or rather the sieve-plate reticulum, at first shows a composition similar to that of the lateral walls of the tube, but in the mature condition its free surface has become converted into callus, so that in a transverse section of the plate there is seen to be a central core of cellulose with a border of callus, whilst in a surface view the pores of the sieve-plate show a callus lining (cf. Text-figs. 2 and 3). A somewhat similarly complex structure obtains in the lateral thread groups, for callus is nearly always to be found in association with the connexions between two adjoining sieve-tubes.

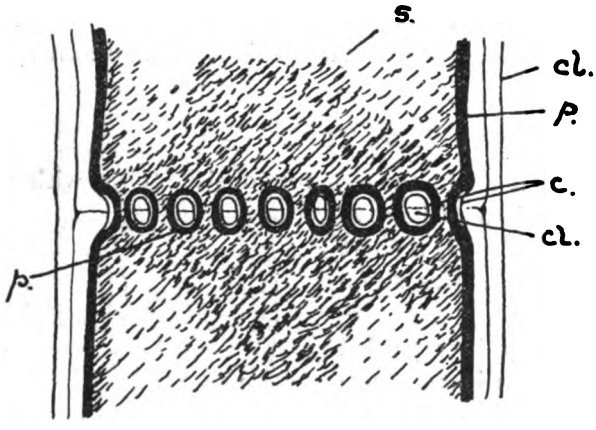

FIG. 2. Diagrammatic longitudinal section of an active sieve-tube of *Vitis*, showing the cellulose wall and meshwork of the sieve-plate in section (*cl.*). The protoplasmic bag (*p.*) encloses the mucilage or slime (*s.*): the sieve-plate meshwork is coated with a layer of callus (*c.*), and this in its turn is separated from the sieve-tube contents by the protoplasm. Cf. the surface view of a similar sieve-plate (Fig. 3).

A transverse section then of a sieve-plate with its slime-strings would show a highly complex structure, for, taking a single string and its pores, there would be in the centre the slime itself, around which is the protoplasmic tubule, and immediately outside comes the ring of callus due to the peripheral alteration of the cellulose membrane of the sieve-plate itself. Such a structure appears to be typical for the sieve-plates of all Dicotyledons, including *Viscum*, though according to Kuhla's figures[1] it possesses no callus in its sieve-plate.

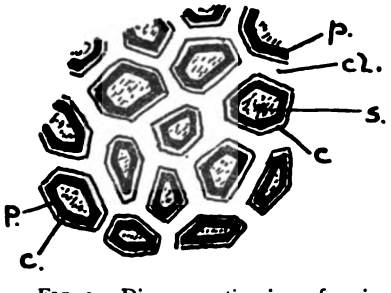

FIG. 3. Diagrammatic view of a sieve-plate similar to that of Fig. 1, in surface view. The mesh of the plate (*cl.*), with the pores lined by callus (*c.*), are shown, through which the slime-strings (*s.*) pass, ensheathed by protoplasm (*p.*).

With regard to the position of the sieve-plate, enough has been written by Wilhelm and Lecomte to render any lengthy description unnecessary. The sieve-plates are commonly placed transversely like ordinary end-walls, and they occur in this manner in *Wistaria*, *Cucurbita*, *Viscum*, and in the

[1] Kuhla, l. c., Pl. III, Fig. 17.

sieve-tubes of the protophloem of *Vitis*. In the secondary phloem of *Vitis* and many other plants the end-walls are inclined and bear several sieve-plates,[1] so that in a transverse section the sieve-plates appear to be borne on the radial walls of the tubes.

PHLOEM: GENERAL ANATOMY.

The general anatomy of the phloem varies considerably in different plants. In *Vitis*, which has been so carefully studied by Wilhelm, and also in *Tilia* (cf. Perrot, pp. 127, 128), the phloem is composed of alternating bands of sieve-tubes, together with their companion cells and parenchyma, and of bast fibres.[2] In *Wistaria* the alternation of fibres amongst the

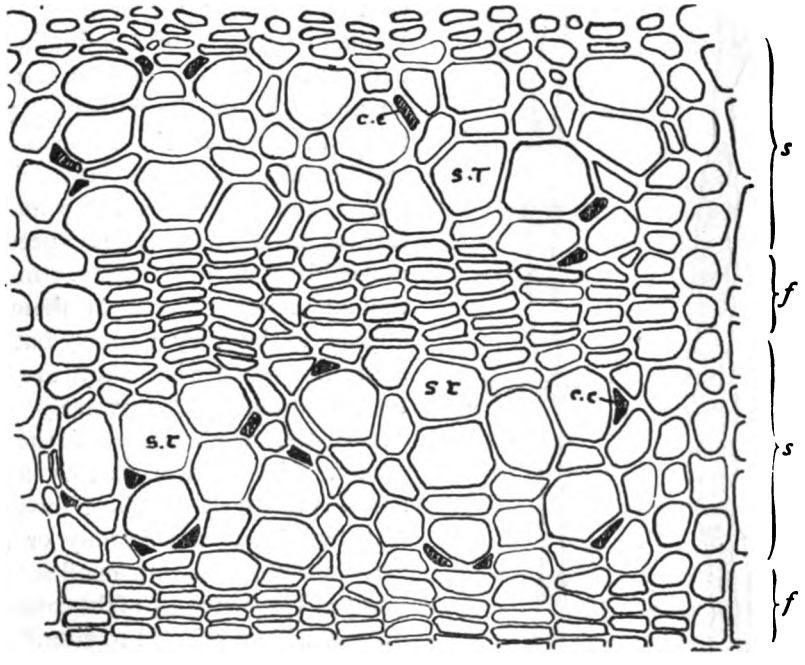

FIG. 4. *Vitis*. Phloem in transverse section, showing the alternating bands (*s*.) of the sieve-tubes (*s. t.*) and companion cells (*c. c.*), and the bast fibres (*f.*); the edge of a medullary ray is seen on the right. (Copied from Wilhelm, Pl. I, Fig. 1.)

sieve-tubes is less regular, but the latter show an interesting feature in always having their sieve-plates at about the same horizontal levels. This characteristic makes *Wistaria* a useful plant for research, since a single favourable transverse section will show sieve-plates in every stage of development. *Viscum* is peculiar in that its sieve-tubes and companion cells are grouped in lenticular areas, as seen in a transverse section, which

[1] Wilhelm, l. c., Pl. II, Fig. 16, &c.; Perrot, l. c., Fig. 39.

[2] Ibid., Pl. I, Fig. 1.

are enclosed like small islands in the general parenchymatous tissue (Fig. 27, Pl. XVII). *Viscum* also differs markedly from the other plants which have been examined, both in the form and position of the companion cells, and also in the distribution and character of the connecting-threads between them and sieve-tubes, and in the lateral walls between adjoining sieve-tubes.

The Companion Cells.

The anatomical relation of the companion cell to the sieve-tube is too well known to need any remark. In the case of *Vitis*, excellent figures are given by Wilhelm[1] (cf. Text-figs. 1 and 4), and there is also a short résumé of the subject by Perrot.[2] Two of Wilhelm's figures of longitudinal sections are of especial interest (viz., Figs. 26 and 35), for the numerous pits in the wall between the companion cell and its sieve-tube are very faithfully drawn, and the addition of connecting-threads in the pits is only needed to make his picture complete. In longitudinal section the pits in these walls appear to be very narrow, but when seen in surface view they are found to be extended transversely almost across the whole face of the companion cell to form narrow fusiform areas, over which the short threads are distributed either uniformly or aggregated more or less into small secondary groups. The histology of the connecting-threads will be referred to later.[3]

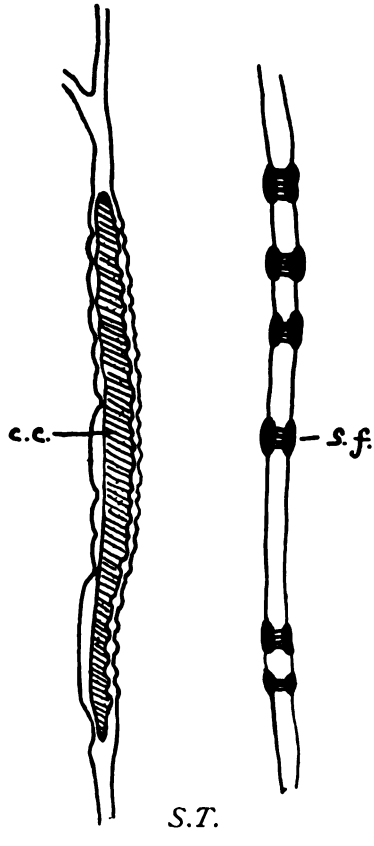

Fig. 5. *Vitis.* A sieve-tube (*S.T.*) and companion cell (*c.c.*) in tangential section. The radial wall of the sieve-tube shows several sieve-fields (*s.f.*) in the winter condition, with callus-rods crossing the wall, and small callus-cushions. (Copied from Wilhelm, Pl. IV, Fig. 35.)

The Bast Parenchyma Cells.

Between the bast parenchyma cells and both the sieve-tubes and the companion cells there are small pits of connecting-threads similar in character to those which connect the cells of the bast parenchyma themselves. The pits are more or less circular in outline

[1] Wilhelm, l. c., Pls. I and II, Figs. 13, 16, 18–20; Pl. III, Fig. 26; Pl. IV, Fig. 35.
[2] Cf. Perrot, l. c., p. 62.
[3] Cf. p. 279.

and fairly deep, and the pit-closing membrane is pierced by a small group of some five to seven threads, as seen in section, which usually stain darkly.

Between a sieve-tube and an adjoining bast parenchyma cell only a few such pits will be seen, though in a single sieve-tube of *Vitis* as many as ten little pits have been counted. In winter material small hemispherical pads of callus are found covering these pits on the sieve-tube side, and with appropriate treatment the threads or their remains can be seen fanning out to the margins of these little callus cushions (v. Fig. 49, Pl. XVIII).

Between the bast parenchyma cells and companion cells small pits of threads are fairly numerous, and appear to be similar in every respect to those between two parenchymatous cells,[1] and no callus is formed over them in winter material. As was noticed in the case of *Pinus*,[2] callus is formed normally only in the sieve-tubes themselves.

The pits in the walls of the phloem medullary ray cells are very numerous, and are occupied by small groups of threads similar to those of the phloem parenchyma and of parenchymatous tissue generally.

Bast Fibres.

Bast Fibres are a further constituent of the phloem commonly to be found, and may occur in definite bands alternating with the bands of sieve-tubes, as in *Vitis* (cf. Text-fig. 4) and in *Tilia*, or with a somewhat irregular distribution as in *Wistaria*. In *Viscum* and *Cucurbita* bast fibres are apparently absent.[3]

When young their protoplasmic contents are in communication by means of connecting-threads, both amongst themselves and also with adjoining parenchymatous tissue. In *Vitis* the gradual development of the thick cell-walls has been followed; the final result is the formation of deep funnel-shaped pits with thin pit-closing membranes, which are traversed by a number of short and delicate threads (Fig. 53, Pl. XVIII). As long as the walls of these sclerous cells are increasing in thickness they retain their protoplasmic contents and their connecting-threads, but on the completion of the secondary changes they become dead and empty, and the perforations of the membranes either remain as empty holes or, more probably, are obliterated.

THE DEVELOPMENT OF THE SIEVE-FIELDS AND OF THE SIEVE-PLATES.

The Sieve-Fields.

The term 'Sieve Fields', in the sense in which it is used in this paper,[4] denotes the groups of fine connecting-threads or strings which are found normally on the lateral walls, and which serve as a means of communication

[1] Wilhelm, l. c., Pl. IV, Fig. 36.
[2] Hill, Ann. Bot., vol. xv, p. 597, &c.
[3] *v.* Perrot, pp. 76 et seq., and Figs. 59 and 60.
[4] *v.* p. 251, footnote 6.

between adjoining sieve-tubes. The sieve-plates occur on the horizontal or oblique end-walls of the sieve-tubes, and occasionally on the lateral walls also, but their slime-strings are readily distinguished from those of the sieve-fields owing to their larger size. As the developmental history of the fields appears to be simpler than that of the plates, it may help towards a better understanding of the latter structures if the details of sieve-field development are first considered.

Great difficulty has been experienced in trying to demonstrate threads in tissues arising from the cambium or in any embryonic tissue, and the youngest sieve-tube in which threads can be seen in the lateral walls is at some distance from the actual cambium, and removed, in the radial direction, by not more than two or three cells from the youngest active sieve-tube. That threads have not been successfully seen as yet in the youngest cell-walls is due probably to the limitations of our methods rather than to their absence. The walls of the very young elements cut off from the cambium are extremely thin, and of course contain a high percentage of water, so that it seems likely that they undergo relatively violent alterations on fixation, and that the demonstration of connecting-threads is thereby prevented. Similar distortion of cell-wall structure appears to take place with the fixation of mature tissues in Alcohol, since if this reagent is used even with full-grown walls it is impossible as a rule to see anything of the finer histological details.

It is not until the walls of the young elements show a distinct and definite increase in thickness, somewhat like that described for the phloem of *Pinus*,[1] that it is possible to stain the fine protoplasmic threads. In the case of *Vitis* a differentiation of the lateral walls of the sieve-tubes is soon noticeable, giving rise to shallow pits separated by thicker portions of the cell-wall. In a transverse section these pits are often seen to be considerably elongated in the transverse direction, and in a surface view they are more or less elliptical or fusiform in outline, and are closely crowded over the surface of the wall.[2] The thin pit-closing membranes are provided with delicate, faintly-staining threads, which in a surface view are seen to be distributed more or less evenly over the surface. The threads appear to be continuous across the membrane, and there is no evidence of any break or node at the middle lamella (Fig. 37, Pl. XVIII).

In a slightly older sieve-tube there is a noticeable increase in the thickness of the wall, leading to a greater distinction of the pitted areas; the threads, which still present an unbroken appearance, are somewhat longer and more prominent than before, but show a tendency to become arranged in small secondary groups within the limits of the original pit. On examining a pit such as that just described, after the action of Water

[1] Hill, Histology of Sieve-Tubes of *Pinus*. Ann. Bot., vol. xv, p. 587, 'The boundary layer.'

[2] Cf. Wilhelm, l. c., Figs. 29 and 38.

Blue, tiny specks or dots of callus will probably be seen at the end of each thread on one side of the membrane, or possibly on both surfaces (Fig. 38, Pl. XVIII). With increasing age the thickened portions of the wall become more conspicuous, and the smaller groups of threads also tend to become arranged in small secondary pits (Fig. 39, Pl. XVIII). Whilst this change in the general configuration of the cell-walls has been taking place, more remarkable changes are taking place in the character of the threads, and in the wall in their immediate environment. The callus has now reached the middle lamella, usually at first from one side only (Fig. 39, Pl. XVIII), and then shortly afterwards from the adjoining element, so that each thread shows a callus coating (appearing as a blue rod with Water Blue alone), which has been formed by an alteration of the cell-walls of the two sieve-tubes concerned, proceeding inwards as far as the middle lamella (Fig. 40, Pl. XVIII).

By slightly swelling the walls and treating the section with either Russow's Callus Reagent or Water Blue, the callus-rods, which appear at first sight to be continuous across the membrane, are seen to be made up of two distinct portions or pegs belonging to the walls of their respective sieve-tubes, and apparently there is no fusion or actual contact between them (cf. Figs. 12 and 13, Pl. XVII).

With the appearance of the callus-rods a further structure becomes visible, namely, a median node or dot, situated between the two opposed pegs of the callus-rod, which is stained with protoplasmic dyes (Fig. 16, Pl. XVII). The formation of the callus-rods is, however, only the obvious sign of a more important change, which has taken place in the character of the threads themselves. Ferments—which, as will be shown later, also determine the structure of the developing sieve-plate—appear to attack the fine threads of these lateral sieve-fields, and bore out the holes or capillaries in the cell-wall through which they pass from cell to cell.[1] So then not only do the ferments, which pass in from each sieve-tube, enlarge the thread canals, but they also alter the nature of the wall in the immediate neighbourhood, by converting it into callus as far as the middle lamella. The contents of these fine tubes are found to react in a different manner to stains after the appearance of the callus, for the protoplasmic threads of the embryonic condition have now become the more important slime-strings of the mature sieve-tube, and in addition to this the small darkly staining node has been formed at the middle lamella. Each slime-string is thus seen to be contained in its own callus-rod or tube, and there is no such inclusion of several strings in a single callus 'cork' as has been described for *Pinus*[2] and other Gymnosperms. In *Wistaria* the development of the

[1] Cf. Hill, l. c., p. 588. The probable rôle of ferment action in the production of the slime-strings and callus-rods was suggested by Gardiner, and he drew my attention to the analogous appearances presented by the endosperm walls of *Tamus communis* during germination.

[2] Ibid., p. 590.

fields is easier to study than in *Vitis*, since the threads are fewer, larger, and well separated from each other. In a surface view of the wall of a young sieve-tube, the individual strings, each enclosed in its callus-sheath, can be clearly seen (Fig. 15, Pl. XVII), whilst in a section the paired callus-rods with their contained slime-string, together with the node at the middle lamella, are no less conspicuous after treatment with Safranin and Water Blue (Fig. 14, Pl. XVII).

Precisely similar courses of development appear to obtain for the lateral sieves of *Tilia*, *Cucurbita*, and other Angiosperms. In *Cucurbita* the development of the callus-rods and the appearance of the median node are very clear, for the young sieve-field, viewed in section, is seen to be studded with little knobs of callus at the surface of each fine thread (Fig. 25, Pl. XVII).

Lateral sieve-fields may be found both in the radial and the tangential walls, and are usually distributed in a similar manner in either wall, but in *Tilia*, and probably in some other plants, the threads are far more abundant in the tangential than in the radial walls (Fig. 59, Pl. XVIII), in some cases being so numerous that a surface view shows the tangential wall covered with dots (Fig. 61, Pl. XVIII). In general structure these threads appear to be similar to those of the smaller groups in the radial walls, but owing to their close crowding it is not easy to determine the exact relations of the callus to the individual threads.

Viscum differs from what appears to be the normal arrangement in some interesting details; the threads in the walls between two sieve-tubes are seen in surface view to be grouped in narrow fusiform areas, with which the walls are closely packed, and the wall appears to be full of threads. In section the threads are seen to be in small pits in groups of three to five, and neither in summer nor in winter can any callus be demonstrated in these lateral walls. The threads do not stain very deeply, and there is no appearance of a node at the middle lamella[1] (Figs. 26 and 27, Pl. XVII).

The Median Node.

If these observations are correct, it seems probable that the conspicuous node at the lamella is in some way a function of the slime-string and callus-rod, since in *Viscum*, where the lateral threads do not become converted into slime-strings, the node is apparently absent.

A slight enlargement at the middle lamella is often noticeable on the threads of ordinary cortical cells, e.g. in the cortex of *Cucurbita*, or on the threads of the thick cell-walls of Endosperms,[2] but in such cases the staining of the thread throughout is of the same character, and the minute

[1] Kuhla figures a node on all these threads. Bot. Zeit., lviii, 1900, Pl. III, Figs. 12–14 and 17.

[2] Gardiner and Hill, Histology of Endosperms; Proc. Camb. Phil. Soc., xi, 1902, p. 449, Pl. V, Figs. 13, 15, &c.

nodal swelling appears to belong definitely to the thread itself.[1] The darkly-staining median nodes of the sieve-field slime-strings appear, therefore, to be similar to those described for *Pinus*,[2] and may be due to the action of the ferment on the small portion of the middle lamella between the two opposed callus-rods. It has been noticed that these nodes can be stained with Safranin without previous mordanting, and that the threads themselves remain unstained by this treatment; also after the action of Russow's Iodine and 75 per cent. Sulphuric acid the median nodes stand out as highly refractive granules, arranged along the middle of the middle lamella, when all other structures have vanished (Fig. 43, Pl. XVIII). After two days in 10 per cent. Potash, and subsequent washing in water and weak Hydrochloric acid, the middle lamella nodes had apparently vanished from the sieve-threads in sections of *Vitis*, since they could not be seen after treatment with Russow's Iodine and 75 per cent. Sulphuric acid. With the 'Acid Violet' method of staining, however, the nodes reappear, and it is probable that in this latter case we have to deal simply with a precipitation effect in the empty spaces once occupied by the substance of the nodes themselves. The callus also disappears with the Potash treatment, and the split middle lamella with the thread-tubes in the two separated portions of the wall can be clearly seen (Fig. 42, Pl. XVIII).

Owing to the very small size of the median node, it is by no means easy to obtain a clear idea of its nature, but from the experiments which have been made it appears that this conspicuous structure is a kind of connecting-link between the two callus-rods of a single string containing or enclosing the median portion of the string itself (Fig. 41, Pl. XVIII).

The Sieve-Plates.

The study of the development of the sieve-plates is a matter of greater difficulty than that of the sieve-fields, owing to the extremely delicate character of the membrane which is to become the sieve-plate.

The most common stage of development met with in surface views of properly preserved material shows the young sieve-plate furnished with more or less polygonal areas, which give the callus reaction, separated from each other by narrow bands of cellulose. These cellulose bands, which will constitute the mesh of the adult sieve-plate, appear in section as ridges on either side of the membrane, and it is owing to their elevation above its general surface that the young sieve-plate membrane assumes its deeply pitted character.

The pits are lined by callus in the form of little basins[3] (Figs. 1 and 3, Pl. XVII), but the actual pit-closing membranes, together with the ridges or mesh of the plate, are formed of cellulose (Figs. 1 and 2, Pl. XVII). Younger stages have been observed in which, owing to the shallow pits, the cellulose

[1] Cf. Gardiner on the mode of formation of the initial cell-wall; Proc. Camb. Phil. Soc. xiv, 209.
[2] Hill, l. c., p. 587, Pl. XXXI, Fig. 11.
[3] Cf. Russow, Sitz. d. Nat. Ges. Dorpat, 1882, p. 304.

membrane shows a definitely reticulated structure in surface view before there is any trace of callus formation.

In all cases the protoplasm is seen to have pit processes which occupy each depression of the young sieve-plate (Figs. 2 and 3, Pl. XVII) in a manner precisely similar to that of the pit-fillings of normally pitted tissues. Very rarely traces of delicate connecting-threads have been seen crossing the thin pit-closing membranes of these youngest sieve-plates, and there seems little reason to doubt that they are traversed by groups of fine threads like those seen crossing the pit membranes between other forms of tissue (cf. Text-fig. 6, p. 271). It seems probable that the development of the sieve-plate may not take place along quite the same lines either in different plants or in the same plant at different times of the year.

Wistaria.

Before discussing the nature or cause of this variation, a description of the mode of formation of the sieve-plate in *Wistaria* will be given, since some of the clearest and most definite results have been obtained from the study of this plant. The general appearance of the horizontally placed end-wall of the young sieve-tube—the embryonic sieve-plate—has already been noticed (cf. Fig. 1, Pl. XVII, and Text-fig. 6).

Protoplasmic threads have not been successfully demonstrated in the very youngest stages, and even when the pits of the sieve-plate show their callus linings no certain proof of threads crossing the cellulose membrane has been obtained. There is very good reason, however, to believe that there is a group of protoplasmic threads in each of these membranes, but definite proof of their existence does not seem possible by the methods at present at our command.[1]

In sieve-plates showing the paired callus-basins, the protoplasm of the sieve-tube may be seen forming little rounded pit processes or fillings which fit into the pits of the young plate, and a close examination of these processes when seen in section reveals the presence of little papillae at their base (Figs. 2 and 3, Pl. XVII). The protoplasm of the sieve-tube is always slightly contracted by fixation, and so also are the pit fillings; the appearance just described appears to be due therefore to the retraction of the little papillae from definite holes in the floor of the callus-basins. This view is confirmed by a surface view of a young plate, for a group of small round holes may be seen occupying the central portion of each polygonal callus-area

[1] Strasburger (Jahrb. f. wiss. Bot., Bd. xxxvi), working with Alcohol material, states that he has seen the fine protoplasmic threads in the pit membranes of the very young sieve-plates, and he gives two figures (*v.* Pl. XIV, Figs. 29 and 30) in illustration of his views. It is hardly possible, however, to trace any correspondence between his figures of the surface view and the section of similar sieve-plates, nor do his figures of the next older stage in section and surface view (Figs. 31 and 32) enable one to arrive at a clear idea of his views as to the construction of the young sieve-plate.

(Fig. 4, Pl. XVII, and Text-fig. 7). Into these holes or tubes the papillae of the pit processes fit, and in some instances portions of these papillae may be seen in the actual holes in a surface view of such a plate. Owing to the necessary thickness of the sections it is practically impossible to distinguish these little pores in a transverse section of a sieve-plate, for there must be a certain amount of (blue staining) callus either at the back or the front of a pore by which it is hidden from view. In the next stage the paired pit processes on either side of the thin membrane are found to be united by a group of short darkly-staining threads or slime-strings, and the callus extends across the membrane. In some cases the small strings are very short and

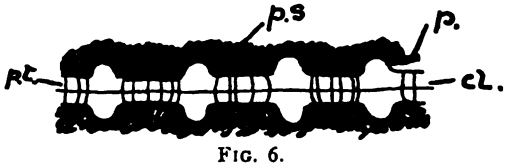

FIG. 6.

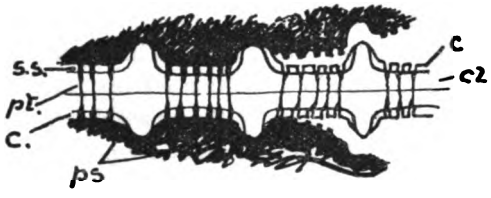

FIG. 7.

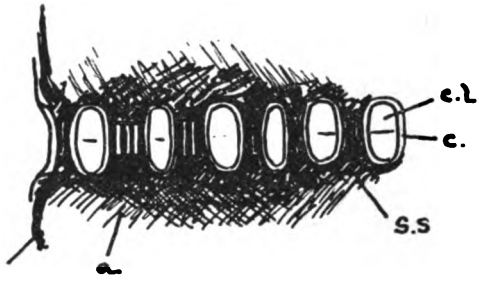

FIG. 8.

Diagrammatic sketch to show the development of the slime-strings of the sieve-plate from groups of threads:—

FIG. 6. Shows the deeply pitted plate or end-wall, with connecting-threads (*p. t.*) crossing the pit-closing membrane (*cl.*) and uniting the protoplasm (*p.*) of adjoining sieve-tubes.

FIG. 7. A later stage; ferment action has commenced, the threads are being bored out to become small slime-strings (*s. s.*), and this is accompanied by the alteration of the surface layers of the membrane (*cl.*) to callus (*c.*). The final stage is the fusion of the small slime-strings to one large string (cf. Figs. 2–4, Pl. XVII).

FIG. 8. The active sieve-plate almost completely developed at *a*; a group of slime-strings is seen crossing the pit-closing membrane, which is now converted entirely into callus (*c.*), whilst at the other end of the plate single thick slime-strings are seen, due to the confluence of the group of strings, each consisting of a mucous string ensheathed in its protoplasmic tube; protoplasm and slime are not differentiated in this figure (cf. Figs. 2 and 3, p. 262, and Figs. 5–9, Pl. XVII).

difficult to distinguish (Fig. 8, Pl. XVII), whilst in others they are of a fair length and the whole group can be seen to be definitely ensheathed in callus (Figs. 6 and 7, Pl. XVII). The mesh of the sieve-plate retains its cellulose character, but is everywhere coated with callus.

These groups of small slime-strings, which on the analogy of the sieve-fields must be due to the boring out and enlargement of pre-existing connecting-threads, have as a rule only an ephemeral existence; for the little membrane, now converted into callus—through which each group passes—is rapidly disorganized, with the result that a large open hole is formed

through the sieve-plate in the position of the original pit, and the group of fine threads is supplanted by the single large slime-string of the active sieve-tube (Text-fig. 8). The characteristic appearance of a sieve, with its more or less polygonal holes of varying size, is thus produced. Each hole has a thin lining of callus, and in section it is seen that the whole of the free surface of the sieve-plate mesh is coated with a thin film of callus (Figs. 8 and 9, Pl. XVII).[1]

By means of the short, thick slime-strings, with their accompanying protoplasm, the sieve-tubes are placed in direct continuity throughout the length of the plant, and may now be considered to be in the active condition (Text-fig. 8). Owing to the rapidity with which the developmental changes proceed, and to our ignorance of the periodicity of any given plant with regard to such changes, the earliest stages of the sieve-plate are unfortunately very rarely met with.

Throughout the summer the holes in the sieve-plate remain fully open, but towards autumn, and occasionally at an earlier period of the year, they become constricted owing to the enlargement of the callus. This may be due partly to the swelling of the callus lining of the tubes, but mainly it is caused by the deposition of fresh callus by the protoplasm on the surface of the plate and of its pores. The slime-strings are thus narrowed and lengthened, with the result that the translocation current through the sieve-plate is gradually inhibited (Fig. 10, Pl. XVII). This process may continue until a considerable accumulation of callus has been formed on either side of the sieve-plate.[2] After a time the slime-strings become disorganized, owing to the accumulation of callus, and the communication between the sieve-tubes ceases, but the much attenuated pores or canals may still be seen crossing the callus-pads, often enclosing disintegrated portions of the slime-strings, whilst at the middle lamella the original cellulose membrane is clearly visible (Fig. 11, Pl. XVII).

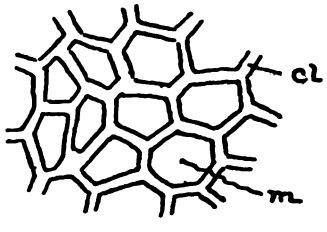

FIG. 9. An old sieve-plate of an empty sieve-tube, in surface view, to compare with Fig. 2. The protoplasm, slime, and callus have vanished, and a cellulose mesh-work (*cl.*) with empty holes (*m.*) is left.

In *Wistaria* the sieve-tubes function for one year only, so that the further history of the sieve-plate simply concerns its degradation. The

[1] Contrast Strasburger, l. c., Pl. XIV, Figs. 33 and 34. According to Strasburger the boring of the sieve-areas commences by a small hole after the fusion together of the callus-rods which have arisen by the transformation of the protoplasmic threads.

The view here put forward, however, entirely differs from this, since there seems good evidence to prove that the largest possible hole is formed in each area immediately on the fusion or boring out of the group of small slime-strings, which are the transformed protoplasmic threads.

[2] As has been noticed by Lecomte, l. c., p. 265, the callus-cushion may be unequally developed on the two sides of a sieve-plate.

callus is dissolved away by ferments, and only the cellulose mesh or reticulum of the plate is left (Text-fig. 9).[1]

Cucurbita.

The course of sieve-plate development described for *Wistaria* appears to obtain in *Cucurbita* also. The sieve-areas are here very large, and when young and only lightly covered by callus show little holes as though they had been pierced by pins. In some cases the pit-processes of adjoining sieve-tubes were united by definite threads or fine strings which appeared to pass through the holes (cf. Text-fig. 6).

It is important to point out that the size of the individual sieve-areas or pits varies considerably in any sieve-plate; those near the centre being larger as a rule than those near the periphery. It naturally follows that the smaller the area the fewer will be the threads which it contains, and in fact in the case of some of the smallest of these areas there seems to be only a single thread. Such cases as these afford a link between the ordinary sieve-fields and the larger sieve-plates, and, moreover, in some plants it appears probable that each area of a sieve-plate is provided with but one thread. The further history of the sieve-plate agrees closely with *Wistaria*. Large holes are formed in the meshes of the plate and there is a thin callus lining to each hole. After a period of full activity callus is gradually laid down over the sieve-plate to form a conspicuous pad, and the slime-strings, though they still remain thick, are considerably lengthened (Fig. 19, Pl. XVII). With the approach of autumn the callus masses are more pronounced, and the slime-strings become attenuated towards the periphery of the callus-cushions, so that each string is somewhat fusiform in shape and a surface view shows a small string in the middle of each callused sieve-area (Figs. 20 and 22, Pl. XVII). Finally, the slime-strings become broken up and cease to function as connexions, though the holes through which they passed still exist as such in the old callus-pads, and with appropriate methods their paths may be seen either as clear lines or more commonly marked by lines of dark granules, representing the remains of the slime-strings (Fig. 21, Pl. XVII).

The presence of pores in the old callus-masses appears to have escaped general notice,[2] for, according to the usually accepted view, the callus covers over and obliterates all trace of the slime-strings on the approach of winter.[3] There is, however, no doubt that the holes or tubes do persist in the oldest plates, and their gradual attenuation seems to form quite a sufficient obstacle to the translocation currents. The importance to the plant of the

[1] Cf. Strasburger in Jahrb. für wiss. Bot., xxxvi, 1901, Pl. XV, Figs. 41 and 42. It is unfortunate that in these, as in some other figures, the proper correspondence between the surface views and sections is not clearly shown.

[2] Cf., however, Lecomte, l. c., p. 266, and Figs. 5, 6, and 13.

[3] Wilhelm, Beit. z. Kennt. d. Siebröhren, Leipzig, 1880, Pl. VII, Figs. 80, 81, 85 &c.; Strasburger, l. c., Pl. XV, Fig. 40.

persistence of these pores in the callus-pads will be the more evident when the question of the re-opening of the sieves after the winter's rest is considered.

VITIS.

The large inclined sieve-plates of *Vitis* which next claim attention are really compound structures, since they are made up of several small plates (Fig. 47, Pl. XVIII), each of which is constituted like the horizontal plates of the sieve-tubes of other plants. Two methods of sieve-plate development appear to obtain in *Vitis*; in the one, the course of events seems to be closely similar to that already described, and distinct indications of little groups of four to five threads have been seen in each sieve-area (Fig. 30, Pl. XVIII). In one instance there was fairly clear evidence of threads in the walls between the paired callus-basins (Fig. 31, Pl. XVIII). In other cases, however, there can be little doubt that there is never more than one thread to each sieve-area, so that each small sieve-plate is quite comparable in its general structure to a sieve-field. Paired knobs of callus, owing to ferment

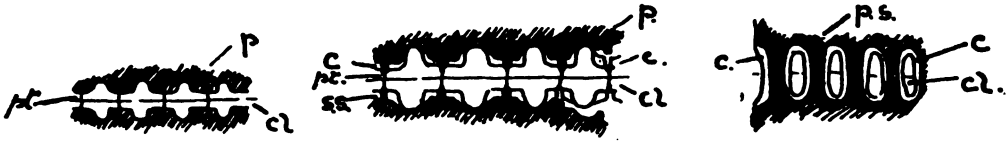

FIG. 10. FIG. 11. FIG. 12.

Diagrammatic sketches to show the development of the slime-strings from single threads :—

FIG. 10. A young pitted sieve-plate (*cl.*), with single threads (*pt.*) in each pit.

FIG. 11. The commencement of ferment action, showing the enlargement of the ends of the threads to form slime-strings (*s. s.*) and the formation of the callus basins (*c.*).

Fig. 12. The final shape, in which the thread has been bored out and enlarged, and the callus change has proceeded right across the membrane and forms a coating (*c.*) to the cellulose sieve-plate (*cl.*).

action, are now formed on either side of the pitted membrane at the ends of each thread, and the change gradually spreads across the membrane until a slime-string in its callus-rod or sheath is formed (Figs. 32 and 33, Pl. XVIII, and Text-figs. 10 and 11). Several cases of a dark dot between the two opposed callus-knobs have been noticed (Fig. 32, Pl. XVIII), which may represent the node of the original thread in course of alteration,[1] the pieces of the slime-string itself having been retracted from the callus owing to imperfect preservation of the material. In surface view such sieves show a small dot in the centre of a small callus-area (cf. Fig. 33, Pl. XVIII, with the sieve-fields of *Wistaria*, Fig. 15, Pl. XVII). The slime-strings so formed soon begin to enlarge, but a swollen node is often seen at the centre of each string, and in such cases the callus does not appear to be con-

[1] Cf. with the node on the threads of the sieve-fields (Figs. 14, 16, 18, Pl. XVII, and Figs. 45 (*b*), 59, Pl. XVIII).

tinuous across the membrane, thus suggesting a further analogy with the sieve-fields (Fig. 34, Pl. XVIII). After a short time, however, the boring out appears to be complete, and a string of equal breadth enclosed by its sheath of callus unites the contents of the adjoining tubes (cf. Figs. 36 and 47, Pl. XVIII). It has been frequently noticed that the pit areas at the extremities of the large inclined sieve-plates of *Vitis* are occupied by threads of the definite sieve-field type, having median nodes and small callus-rods exactly like those on the lateral walls (Fig. 46, Pl. XVIII). Such observations are not without interest in connexion with the analogy which has been shown to exist between these two forms of connexions.

Further, the sieve-fields may often undergo a subsequent boring after they have reached their normal stage of development. The pores are widened apparently by renewed ferment activity and the median nodal structure vanishes (Figs. 45 and 48, Pl. XVIII), so that to all intents and purposes a small sieve-plate has been developed as a secondary formation from a sieve-field. All these cases lend colour to the view that the sieve-fields and sieve-plates undergo similar developmental stages, but that in the case of the latter structures the changes have been more far-reaching.

The gradual accumulation of callus on both sides of the sieve-plates in *Vitis* takes place in the manner already described for *Wistaria* or *Cucurbita*, and the paths of the slime-strings are left as fine tubes after the disorganization of the strings themselves has taken place, i.e., when the slime disappears from the tubes on the approach of the resting period (Fig. 54, Pl. XVIII).

Good figures of the sieve-plates in the winter condition are given by Wilhelm,[1] and in two cases (Pl. VII, Figs. 89 and 90) he shows lines crossing the callus-masses. As the material was collected on April 27, these lines may represent the new slime-strings in the old pores of the callus-masses.[2]

Re-opening of the Sieve-Plates in *Vitis*.

Vitis differs from the plants already mentioned in that its sieve-tubes are functional for more than one year. The sieve-plates, therefore, which become blocked by the callus in the ordinary manner in the autumn have to be reopened in the course of the coming spring. In order to obtain evidence of the time and mode of reopening of the plates, two plants of *Vitis* of different varieties were examined by means of sections taken at short intervals of time from February until the end of May in 1901, and some similar observations were made in 1904. The plants were grown in the open at the Cambridge Botanic Garden. It was noticed that at the beginning of April the buds began to swell, and on cutting into the stem the vine bled. The sieve-tubes and callus-cushions, however, appeared to be in the

[1] Wilhelm, l. c., p. 37.

[2] Perrot, Le Tissu criblé, 1899, Figs. 20, 21, and 32, copied from Lecomte.

same condition as in February and March, for the callus showed sharp contours, and no slime-strings were present. The pores of the old strings were seen only with difficulty and often appeared as dotted lines crossing the callus owing to the remains of the disintegrated portion of the slime-strings (Fig. 54, Pl. XVIII). By about April 20 the phloem again became active and the slime reappeared in the sieve-tubes and began to force its way through the old pores in the callus-masses. The callus had as yet undergone scarcely any change and preserved a firm and definite outline (Figs. 55 and 56, Pl. XVIII). By the beginning of May a great change was found to have taken place: the slime-strings had all been re-established and the callus had lost its sharp outlines and would not stain very deeply with the usual reagents, owing no doubt to the action of the ferments, which were actively engaged in its solution (Fig. 57, Pl. XVIII). At the end of May the sieve-plates were found to be practically in the normal summer condition, with the callus forming a delicate lining to the pores through which the short thick slime-strings passed (cf. Fig. 9, Pl. XVII, and Text-fig. 8, *s.s*).[1]

Sieve-Tubes crossing the Medullary Rays.

Wilhelm[2] was the first to describe and figure the short cross-connecting sieve-tubes in *Vitis vinifera*, which traverse the medullary rays and join one group of sieve-tubes with another.

Similar anastomosing strings of sieve-tubes were noticed by Russow in *Quercus pedunculata*. Those of *Vitis* are again described and figured by Lecomte,[3] whose figure is reproduced in Perrot's treatise.[4]

These cross-connecting bands or strings of sieve-tubes are composed of small elements, each being about the size of the adjacent parenchymatous cells, and there seems every reason to believe that they represent ordinary medullary-ray cells, which have undergone special development (Text-fig. 13).

It is unfortunate that, owing to the difficulty of finding these cross-connexions in sections of young material, their development has not been able to be studied in detail; for it seems probable that interesting information might be obtained as to the formation of slime-strings from the original thread-groups of the parenchymatous cells which serve as the mother-cells of these short sieve-tubes and companion cells.

The sieve-plates of these sieve-cells are essentially similar to those of the ordinary sieve-tubes, and show well-developed callus-pads.

[1] At the beginning of April the winter buds had begun to swell, and the Vine bled on being cut. Towards the end of the month the buds were bursting, and by May 9 the young shoots were one to two inches in length, and there was no 'bleeding' of the Vine on cutting. On May 16 one of the leaves of the young shoot had unfolded. Root pressure becomes active some three or four weeks before any visible changes take place in the phloem.

[2] Wilhelm, l. c., Pl. VI, Fig. 69.

[3] Lecomte, l. c., Pl. XXIII, Fig. 40.

[4] Perrot, Le Tissu criblé, p. 42, Fig. 24.

Companion cells, either one or two, accompany each sieve-cell and illustrate clearly the way in which both they and the sieve-tube are formed from the same mother cell. The wall between sieve-tube and companion cell is crowded with threads as is the case in the phloem proper, and their distribution and character in such walls suggest that the threads are closely connected with the processes of cell division.[1]

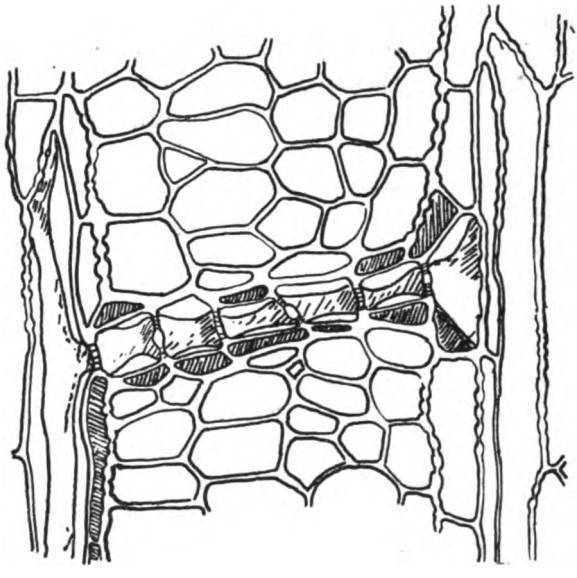

FIG. 13. *Vitis*. Tangential section showing a string of sieve-tubes crossing a medullary ray. The connecting slime-strings crossing the callused sieve-plates are well seen. The short sieve-tubes are accompanied by their companion cells. (Copied from Wilhelm, Pl. VI, Fig. 69.)

THE CALLUS.

An examination of a callus-pad which is being reopened, and from which the slime-strings have been removed, throws some light on the way in which these masses are built up. For owing to the swelling action of the ferment a very regular and definite stratification is disclosed, which lends support to the view that the callus-cushions or pads are laid down by the protoplasm in definite layers in a manner similar to that of the formation by apposition of ordinary cell-walls. It seems highly probable, therefore, that the protoplasm has the power to deposit callus as it would cellulose in accordance with the demands of the plant (Fig. 58, Pl. XVIII). Additional evidence is afforded by those rare cases in which a layer of callus is laid down all over the wall of the sieve-tube apparently in place of a layer of cellulose such as that seen and figured in *Phaseolus* (Fig. 28, Pl. XVII).

[1] Cf. Gardiner, Roy. Soc. Proc., 1900, p. 186, and Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

As the question of callus formation has been discussed somewhat fully in an earlier paper[1], there is no need to enter upon the subject again in any great detail. This research on the Angiosperms has tended to confirm the results already obtained, and there seems now little reason to doubt that the earliest formation of callus, which is coincident with the development of the slime-strings, is due to the alteration of pre-existing cellulose, whilst the superficial pads or cushions of the later-formed or secondary callus are gradually deposited by the protoplasm.

Some account of the diverse views on callus formation has also been given in the introductory portion of this paper, and Russow's interpretation of the shape of the callus-basins was mentioned. He considered that the polygonal outline of these basins or pits—as seen in a surface view of a young plate—afforded definite proof that the callus is laid down entirely by the protoplasm. There seems, however, very little reason for believing that the shape of these areas necessarily throws any light on their mode of formation. The pits of the young sieve-plate are at first more or less circular, then, as the whole membrane grows, they increase considerably in size, until finally the mesh or framework of the sieve-plate represents only a small proportion of the total area. As these changes proceed, the concomitant growth naturally causes the pitted areas to assume an angular outline in consequence of their mutual pressure (cf. Figs. 3, 4, 9, and 22, Pl. XVII).

It is at this stage, when the ground plan of the young plate has become fixed, that the callus makes its first appearance, and it appears to be due to the alteration of the surface layers of these polygonal pit membranes rather than to deposition by the protoplasm (cf. Figs. 1 and 2, Pl. XVII, and Text-figs. 7 and 11).

This view of the primary formation of the callus is also supported by the developmental history of the sieve-fields, where the callus appears as little dots or spherical heads round about the end of each connecting-thread, which, when closely crowded, often assume an angular outline. There seems, therefore, to be little reason to doubt that in all cases the first formation of callus is due to the alteration of cellulose, by the action of ferments, in the immediate vicinity of the connecting-threads (Figs. 38–40, Pl. XVIII).

The Connexions between Sieve-Tubes and other Tissues in the Phloem.

Reference has already been made to the companion cells[2] in their relation to the sieve-tubes, but nothing has been said about the character of the threads connecting these two elements.

The simplest case is afforded by *Viscum*, where the connecting-threads in the walls between companion cells and sieve-tubes appear to be exactly similar to those in the lateral walls between two sieve-tubes. In both cases

[1] Hill, Ann. Bot., vol. xv, pp. 597–602, 605–606.

[2] Cf. p. 264.

the threads are very numerous and are arranged in small groups in pits distributed all over the wall, but at no time does there appear to be any callus associated with the threads. The cell-walls, however, are somewhat mucilaginous and respond readily to swelling agents (Fig. 27, Pl. XVII).

In the case of the more typical Angiosperms the connexions between a sieve-tube and a companion cell are very different in character to those of the sieve-fields. In the latter, the connexions are fine slime-strings ensheathed in callus-rods, and each half of the common cell-wall between the two tubes is of the same nature (Fig. 14, Pl. XVII). But in the case of the wall between a companion cell and a sieve-tube the two portions of the dividing wall show a different composition. On the sieve-tube side of the lamella the wall is fairly broad, whilst on the companion-cell side it is narrow and appears to be somewhat different chemically from the walls of the sieve-tubes. The middle lamella thus comes to occupy an unsymmetrical or extra-median position (Fig. 60, Pl. XVIII).

The connexions between sieve-tubes and companion cells are in consequence also unsymmetrical in appearance, and moreover are found to be composed of two distinct portions. In the sieve-tube 'half' of the wall the threads appear to be like those of the sieve-fields, being accompanied by callus, which reaches as far as the lamella; whilst in the companion-cell 'half' of the wall the threads are necessarily very short and are more like those in the walls of ordinary parenchyma cells, and there is no callus. The condition of affairs is thus analogous to that described for the Albuminous cells of *Pinus*,[1] where each individual 'thread' is similarly made up of two distinct portions. The character of the connexions between sieve-tubes and companion cells in Angiosperms is not nearly so easy of elucidation as is that of the similar connexions between the sieve-tubes and albuminous cells of the Gymnosperms, owing to the thinness of the walls in Angiosperms, and some difficulty exists as to the exact nature of the relation of the callus to the threads. During the spring and summer it is not possible in many cases to demonstrate callus at all in such a position, but in some plants a definite occurrence of callus in the sieve-tube portion of the common wall cannot be doubted (Fig. 29, Pl. XVII, Figs. 51, 52, and 60, Pl. XVIII).

Callus has been seen to extend as far as the middle lamella in winter material of *Vitis* (Fig. 52, Pl. XVIII), but owing to the delicacy of the walls it is very difficult to make out its precise relation to the threads. It seems probable, however, that both in *Vitis* and *Tilia*, and perhaps also in most of the Angiosperms, each whole 'connecting-thread' in the mature condition consists of a slime-string ensheathed in its callus-rod in the sieve-tube portion of the wall, which is then continued as, or rather is in contact with, an ordinary

[1] Cf. Hill, Ann. Bot., vol. xv, 1901, p. 600, Fig. 12, Pl. XXXI; Fig. 12, Pl. XXXII; Fig. 23, Pl. XXXIII.

protoplasmic thread in the wall of the companion cell (cf. Fig. 29, Pl. XVII, and Fig. 60, Pl. XVIII). Some uncertainty also exists as to the presence or absence of a definite median node.

The case of the thread groups between sieve-tubes and bast parenchyma cells appears to be similar to that of the companion cells just described. Here the thread groups show definite callus-cushions in the winter material on the sieve-tube side of the wall (Fig. 49, Pl. XVIII), and in some cases there is clear indication that callus-rods extend through the sieve-tube portion of the wall as far as the lamella (Fig. 50, Pl. XVIII). No callus is formed in the parenchymatous cell, so that here again the threads which appear homogeneous are compounded of two distinct portions—the slime-string in its callus-rod in the sieve-tube portion of the wall, and the protoplasmic thread in the wall of the parenchymatous cell.

At first, no doubt, the threads were protoplasmic throughout, as is the case with the connexions of the sieve-fields, but owing to ferment action the halves of the threads in the sieve-tube portions of the walls undergo secondary modifications and are converted into slime-strings enclosed in callus-rods.

Owing to the difficulty experienced in the staining of the slime-strings in the sieve-tube side of the wall, it is not easy to determine the exact structure of the connexions between the sieve-tubes and these other elements of the phloem; in fact, in most cases the slime-strings appear to have been retracted from the sieve-tube portion of the wall in consequence of the shrinkage on fixation of the general protoplasm. Empty holes are thus left, and it is for this reason, no doubt, that the threads between sieve-tubes and companion cells usually appear to be so short and darkly stained, since, as a rule, only the portions of the threads in the companion-cell half of the wall are visible (Figs. 50–52, Pl. XVIII).

The development of callus in connexion with companion-cell threads is nearly always very slight and difficult of observation, but conspicuous, though small callus-pads are frequently seen over the groups of threads between the sieve-tubes and bast parenchyma cells (Figs. 49 and 52, Pl. XVIII).

The presence of nodes at the lamella, comparable to those found in the sieve-fields, has not been satisfactorily proved, though they appear to be present in the walls between sieve-tubes and bast parenchyma cells (Fig. 50, Pl. XVIII).

General Considerations.

These curious, unsymmetrical thread groups, considered in connexion with the stages of development passed through by the threads of the sieve-fields, suggest questions of considerable interest, both as to the chemical composition of the cell-walls in which they are found and as to the physiological

processes which bring about the secondary modifications of the threads themselves. The real nature of continuity also requires further consideration in the light of the facts which have been detailed.

It is important, in the first place, to notice that the callus in the phloem is found only in the sieve-tubes, and that its inception appears to be due to the action of ferments on the cellulose walls in the immediate neighbourhood of the threads. From what has been said above (cf. pp. 267 and 279), it is clear that the influence of any developing sieve-tube does not extend beyond the actual limits of that element, that is as far as the middle lamella in any direction, for the callus formation accompanying the boring out of the threads always commences at the inner surface of the wall and proceeds outwards as far as the lamella. No cases have been observed of the ferment action from one tube passing across the lamella into the next, but each element behaves as though it is an isolated and independent organism, and the formation of the active slime-strings of a sieve-field is due to influences emanating from two different and distinct sieve-tubes. The unsymmetrical thread-groups between the sieve-tubes and the companion cells and parenchyma cells respectively are thus easy of explanation. For since the sieve-tube influence cannot be exerted beyond the lamella, the original protoplasmic threads remain more or less unaltered in the walls of the cells (companion or bast-parenchyma cells) to which they belong.

What, then, is the nature of continuity of protoplasm? Does the middle lamella, representing the original wall laid down after nuclear division, form a delicate imperforate membrane against which the ends of the threads impinge coincidently from the cells on either side,[1] so that, with Strasburger,[2] continuity must be considered as rather apparent than real? Or are the threads really continuous across the lamella from cell to cell?

The facts of slime-string development appear to lend support to the idea of discontinuity, and it might be held that it is owing to the need of establishing some form of actual continuity that the complex changes, already described, have been evolved.

If this be the case, then true continuity exists only in the phloem, between the sieve-tubes, and possibly between sieve-tubes and the other elements, both companion cells and bast parenchyma cells, with which there are connecting-threads. The connecting-threads in all other—i.e. extra phloem—tissues would, on this view, be little more than very fine pit fillings, separated by a pit-closing membrane formed by the middle lamella.

It is unfortunate that the origin of the threads and their relation to the

[1] The idea of discontinuity was first suggested by Gardiner, v. Roy. Soc. Proc., lxvi, 1900, p. 187. Also cf. Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

[2] Strasburger, Jahrb. f. wiss. Bot., Bd. xxxvi, 1901, pp. 502, 503. It is of interest to notice that A. Meyer, in his review of this paper of Strasburger's, Bot. Zeit., 1902, pp. 102–106, is unable to accept his views on the formation of the connecting-threads. His objections, however, are based on points different to those which have influenced Gardiner in arriving at his conclusions.

cell-plate have not yet been studied in detail, but there seems very good reason for maintaining the views put forward by Gardiner[1] as to the genesis of the connecting-threads. On this hypothesis continuity exists *ab initio*, and at the middle lamella are to be found the first beginnings of the threads, which eventually may become conspicuous as median dots. Certainly the median dot shows the same staining capacity as the rest of the thread, and in many cases it is a scarcely noticeable feature.[2] But whether these nodes of the protoplasmic threads are entire or are bisected by a delicate membrane is somewhat uncertain.

At present, therefore, there seems to be some ground for doubting whether protoplasmic continuity—in the sense of a continuous filament of living protoplasm—exists between vegetable cells.

With regard to the question of the genesis of the connecting-threads, some support to Gardiner's view appears to be given by the distribution and character of the threads in the wall between a sieve-tube and its companion cell. As is well known, these two cells arise by division from a common mother cell, and the wall between them is crowded with threads. When the elements are young, the threads are distributed more or less uniformly throughout the wall, and their arrangement suggests strongly that they have originated in connexion with the processes of nuclear division,[3] and may represent the nodes of the spindle fibres around which it seems probable that the primitive cell-wall is laid down.[4]

With regard to the functions of connecting-threads generally, it seems highly unlikely that they serve for anything more than the conveyance of impulses from cell to cell. Each cell therefore retains its individuality and is master of its own domain, even though it is in sympathetic communication with its neighbours. It is thus conceivable that even if a membrane of extreme tenuity should be found to exist separating the threads of one cell from actual contact with those of another, with which they appear to be in contact, our conception of the efficacy of the threads, as paths for the transmission of stimuli, would not be seriously impaired.[5]

Gardiner's view of the origin of the threads, however, though it has not received definite proof, deserves very careful consideration, since it affords a simple solution of the question of the mode of origin of the threads and one with which it is easy to accommodate the observed facts both as to the

[1] Gardiner, Roy. Soc. Proc., lxvi, 1900, p. 187; Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

[2] Kuhla, Bot. Zeit., 1900, Pl. III. A conspicuous median dot is figured on nearly all the threads. In *Viscum* examined by our methods this dot is seldom seen, and its prominence is probably due to the method employed by Kuhla.

[3] Cf. Hill, Phil. Trans. Roy. Soc., 1901.

[4] Cf. Gardiner, Roy. Soc. Proc., 1900.

[5] Cf. Hill, Ann. Bot., vol. xv, pp. 603-605, where the views of Pfeffer and of Brown and Escombe are discussed.

growth and obliteration of the threads [1] and also as to the secondary changes which they may undergo. It is not easy to understand how Strasburger's plasmodesmic pseudopodia [2] could be formed coincidently on either side of a separating membrane with the regularity with which the connecting-threads are found in the two halves of the common wall.

SUMMARY.

1. The young cell-wall, which will develop into the sieve-plate, is at first a homogeneous, pitted membrane.

2. The pit-closing membranes of the young sieve-plate are crossed either by small groups of fine protoplasmic threads, e. g. *Wistaria chinensis* and *Cucurbita pepo*, or in some cases apparently by only a single thread, e. g. *Vitis vinifera*.

3. Callus makes its appearance in the form of little basins lining the pits of the developing sieve-plate. It appears to be due to the alteration of the superficial layers of the cellulose membrane at these points by ferment action. Eventually this change extends over the surface of the whole membrane.

4. With the inception of the callus-change the fine thread or threads of the young sieve-plate, as the case may be, begin to be bored out to form slime-strings apparently by a ferment, which at the same time affects the pit-closing membrane in the immediate vicinity of the threads and converts it into callus.

5. The enlargement of the slime-strings thus formed continues, with the final result that a single large slime-string is found occupying the place of each pit of the young sieve-plate. In the one case the single string is due to the enlargement and fusion of a group of strings, in the other to the enlargement of a single string.

6. The slime-string is in all cases enclosed in a protoplasmic tube, which passes through the callus-lined pore of the sieve-plate.

7. Subsequent formation of callus appears to be due to protoplasmic activity and not to cellulose changes. The callus is laid down over the sieve-plate by the protoplasm, apparently in definite layers, after the manner of cellulose, and at length so constricts the pores of the sieve-plate that translocation is prevented.

8. The sieve-pores in a large callus-mass are not obliterated, though much attenuated. In the plants examined, whose sieve-tubes function for

[1] Gardiner, Proc. Roy. Soc., 1900, p. 187. Also Hill, Phil. Trans. Roy. Soc., cf. Pl. XXXI, Figs. 1 and 7; Pl. XXXIII, Figs. 21 and 23. Gardiner, Proc. Camb. Phil. Soc., 1907, p. 209.

[2] Strasburger, l. c., p. 503, considers that this meeting of the plasmodesma is no more strange than the formation of corresponding pits. But the formation of the corresponding pits is surely due to the fact that there are already groups of threads at these spots, and that it is necessary for the greater efficiency of the threads that the pit-closing membrane should be thin.

more than one year, the slime-strings are re-formed in the spring along the old paths by renewed ferment activity. The accumulated callus is dissolved away and the plate resumes its normally active appearance.

9. With the disuse and death of the sieve-tube the whole of the callus is dissolved and an open sieve remains which represents the cellulose framework or non-pitted portion of the embryonic sieve-plate.

10. The sieve-fields in the lateral walls between two sieve-tubes show a similar origin to that of the sieve-plates. In the adult condition fine slime-strings, each in a callus-rod, cross the pit-closing membrane. In these cases it is probable that ferments from the sieve-tubes on either side of the common membrane, acting along the original protoplasmic threads, convert them into slime-strings and at the same time produce the callus-change in the cellulose immediately contiguous to the threads. In this way the single string in each callus-rod, characteristic of the Angiospermous sieve-fields examined, is produced. In the Gymnosperms examined a group of fine slime-strings was found in each callus-rod.

11. The activities of the sieve-tube contents appear to be limited by the outer boundary of the cell-wall, i. e., the middle lamella, since ferment action is never seen to cross the lamella, but always to proceed as far as this point from each individual tube. It seems likely that similar restrictions may apply to all cells.

12. The callus-rods are not continuous across the common membrane, but are formed of two distinct half-rods, separated by a median node.

13. The median nodes, which are found at the middle lamella separating the opposed halves of the callus-rods of the sieve-fields, appear to be the product of ferment action on the substance of the middle lamella. In *Viscum album*, where in the sieve-fields no callus is formed, the median nodes are also absent.

14. These median nodes enclose the nodules or nodal swellings which represent the points of origin of the protoplasmic threads; but whether the nodal points are halved by a fine separating lamella (thus preventing actual continuity between cells) is uncertain.

15. Callus-pads may be formed over the lateral sieve-fields, apparently by protoplasmic activity, in the same way as over the sieve-plates, and may be re-opened in a similar manner.

16. Callus does not always accompany the threads or strings of the sieve-fields; it is absent from the sieve-fields of the lateral walls in *Viscum album*. An analogous case may be afforded by the albuminous cells in the leaf of *Pinus sylvestris*.

17. Between sieve-tubes and companion cells, thread-groups, comparable to those described between the albuminous cells and the sieve-tubes of the stem of certain Gymnosperms, appear to exist. On the sieve-tube side of the common membrane the threads, at first protoplasmic, are con-

verted into slime-strings associated with callus-rods, but those of the companion-cell wall are continued as ordinary protoplasmic threads.

18. Similar thread-groups are found in the walls between sieve-tubes and bast-parenchyma cells; they are covered by definite callus-pads during the winter, and median nodes appear to be present.

19. The young bast fibres in the phloem of *Vitis vinifera* are in connexion by means of groups of fine protoplasmic threads.

20. The details of sieve-tube histology appear to be in general harmony in the material examined. The formation of slime-strings from protoplasmic threads and their association with callus-rods, as well as the origin of the callus both by transformation of the cellulose and by protoplasmic deposition, are found to proceed along similar lines in representatives of both Angiosperms and Gymnosperms.

21. The secondary changes which take place in the connexions between the sieve-tubes themselves and between sieve-tubes and other elements of the phloem may be explained as follows. In the sieve-tubes there is the need of the formation of definite holes for the translocation of material, and the connecting-threads serve as useful paths along which to work in order to produce tubes of sufficient diameter to enable an actual flow of slime to take place. Then, since the large holes produced are likely to be a source of danger to the plant when translocation diminishes, the callus is utilized to regulate the bore of the tubes, and finally appears to put a stop to translocatory processes altogether.

22. In the case of the threads between the sieve-tubes and other phloem elements, it is no doubt essential that the means of communication should be enlarged to enable the normal physiological processes to take place. And it seems likely that the numerous enlarged communicating channels allow of the passage of secretions into the sieve-tubes from the companion cells.

23. With regard to the physiological function of the threads it appears that they serve primarily for the transmission of stimuli, but that, owing to subsequent modifications in the special cases of the sieve-tubes, they become enlarged and are able to serve secondarily for purposes of translocation.

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EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Mr. A. W. Hill's paper on the Sieve-tubes of Angiosperms.

The lenses used were Swift's $\frac{1}{6}$ and $\frac{1}{12}$ apoc., Zeiss' $\frac{1}{12}$ apoc., with 6, 8, and 12 compensating oculars.

The following lettering is used in the figures: *c* = callus; *p* = protoplasm; *m* = median node; *cl* = cellulose; *bp* = bast parenchyma cell; *st* = sieve-tube; *cc* = companion cell; *r* = radial; *t* = tangential wall.

PLATE XVII (Figs. 1–17).

Wistaria chinensis.

Fig. 1. A young sieve-plate showing the deep pits lined by callus in the form of paired basins. × 1,000.

Fig. 2. A slightly older plate with pit fillings. The papillae, retracted from the holes in the callus, are seen on the lower edge of the pit fillings. × 1,000. (Cf. Text-fig. 7, p. 27).

Fig. 3. A similar plate to Fig. 2 in surface view; the protoplasmic processes filling the pits show traces of the papillae. × 1,000.

Fig. 4. Surface view of part of a young sieve-plate showing a group of holes in each callus-area. In many cases remains of protoplasmic or slime strings are seen in the holes. × 1,000.

Fig. 5. A slightly older plate, in which the central part of the callus-area appears to be undergoing solution to form the hole for the single slime-string. × 1,000.

Fig. 6. A young sieve-plate in section, with groups of slime-strings surrounded by callus. The original cellulose network of the plate is seen. × 1,000.

Fig. 7. A similar plate to Fig. 6, in surface view. The slime has been removed, and empty holes are seen in each callus-area through which the slime-strings passed; one of the plates shows the formation of a single pore from the original group. The cellulose network is deeply shaded. × 1,000. (Cf. Text-fig. 8, p. 271.)

Fig. 8. A sieve-plate showing the formation of single large slime-strings. On the left a large pore has been formed. In the other cases the small strings are on the point of being broken down. The callus extends across the pit-closing membrane. × 1,000.

Fig. 9. Portion of a sieve-plate, which has just been opened, in surface view. The pores have a narrow lining of callus which also spreads all over the free surface of the cellulose meshwork. × 1,000. (Cf. Figs. 4 and 8.)

Fig. 10. An old sieve-plate in section. The callus has increased, and is blocking the sieves by narrowing the pores; the cellulose meshwork is seen at the middle lamella. × 750.

Fig. 11. An old sieve-plate with callus-pad. The callus stains very faintly with water blue, and the pores are marked by dark dots—the remains of the strings. On the lateral wall are two sieve-fields with callus-cushions traversed by slime-string canals. × 750.

Fig. 12. Young sieve-fields in transverse section; the paired callus-rods reach as far as the middle lamella, but do not cross it to come into actual contact with one another. Stained with Russow's callus-reagent. × 1,400.

Fig. 13. A young lateral wall over-swollen to show the callus-rods abutting against, but not crossing, the split middle lamella. Stained with methylene blue and water blue. × 1,000.

Fig. 14. An older lateral wall between two sieve-tubes in longitudinal section, showing three sieve-fields and a single string of another; each callus-rod encloses a slime-string, and there is a conspicuous node at the middle lamella. × 750.

Fig. 15. A similar wall in surface view with five sieve-fields. The fields have only a few slime-strings, and each has its own callus-rod; the fields are in slight pits. × 1,000.

Fig. 16. A slightly older sieve-field showing the slime-strings with conspicuous median nodes. The callus is not indicated. × 1,000.

Fig. 17. An old lateral wall between two sieve-tubes, partly in surface view and partly in optical section. The callus has formed cushions over the fields, and the groups of median nodes are shown. × 1,000.

PLATE XVII (Figs. 18–25).

Cucurbita maxima.

Fig. 18. A transverse section through the phloem of a root, showing two sieve-tubes with sieve-fields in the radial and tangential walls, and companion cells with threads to the sieve-tubes and to the bast parenchyma cells; sieve-tube wall overswollen. The callus is not indicated. × 800.

Fig. 19. A sieve-plate cut obliquely with a considerable accumulation of callus. The slime-strings, though thick, have been drawn out to some length. × 750.

Fig. 20. An older sieve-plate, almost at the close of its active period; the slime-strings are fusiform in shape, having been attenuated owing to the deposition of the callus. × 500.

Fig. 21. An old sieve-plate with a large callus-cushion. The slime-strings have broken down, and lines of dots mark their paths through the callus; only protoplasm is seen in the sieve-tube. × 500.

Fig. 22. An old sieve-plate in optical section. The meshwork of the plate can be seen through the overlying callus. The slime-strings have become very small owing to the filling of the pores with callus. (Cf. Fig. 9.) × 750.

Fig. 23. A very young lateral field with protoplasmic threads. Callus is beginning to be formed on one side and appears as little globular heads. (Somewhat overswollen.) × 750.

Fig. 24. A field slightly older, seen in section and partly in surface view. The callus-change has proceeded further along the threads towards the lamella from the one side, giving the appearance of short rods. Note also the protoplasmic pit-filling, *p*. × 750.

Fig. 25. A young sieve-field, showing callus-formation on both sides of the wall. The median dots are clearly seen at the middle lamella. × 750.

PLATE XVII (Figs. 26–29).

Viscum album.

Fig. 26. A piece of a sieve-tube in longitudinal section, with a sieve-plate crossed by slime-strings. The callus, which is present in the sieve-plate, is not indicated. The lateral wall shows protoplasmic threads in pits connecting two sieve-tubes. There is no callus associated with these threads. × 1,000.

Fig. 27. A patch of sieve-tubes and companion cells in transverse section. The lateral walls are crowded with threads in shallow pits. Groups of threads between two parenchyma cells and between parenchyma cells and a companion cell (*b.p.c.*) are also seen. × 750.

Phaseolus multiflorus.

Fig. 28. A sieve-tube in transverse section showing a layer of callus laid down all over the wall; also a portion of a sieve-field with slime-strings and callus-pads. × 750.

Wistaria chinensis.

Fig. 29. A sieve-tube in transverse section showing a large pad of callus against the wall between the sieve-tube and the companion cell. Winter condition. × 750.

PLATE XVIII (Figs. 30–58).

Vitis vinifera.

Fig. 30. A young sieve-plate in longitudinal section, showing the paired callus-basins almost in contact and traces of threads at their bases. In some cases the large slime-strings have just been formed. An optical section of one of these callus-basins is figured in which four threads are seen. × 1,000.

Fig. 31. A young sieve-plate showing the paired callus-basins with groups of darkly staining threads crossing the membrane between them. × 1,000. (Cf. Text-fig. 7, p. 27.)

Fig. 32. A young sieve-plate with paired callus-basins. There is only a single thread in each pit, which is converted into a slime-string. × 1,000. (Cf. Text-fig. 11, p. 30.)

Fig. 33. A similar plate in surface view, showing a small slime-string in the centre of each callus-area. × 750.

Fig. 34. A young plate of the 'single-thread' type just bored through. The callus of opposed basins is scarcely in contact, and a large median swelling is seen on each string. × 1,000.

Fig. 35. A plate in a slightly older condition; the callus now forms continuous rods across the plate, and the slime-strings are of the same diameter throughout. × 1,000. (Cf. Text-fig. 12, p. 30.)

Fig. 36. A portion of a similar plate in surface view, showing a fairly large slime-string in the centre of each polygonal callus-area. × 1,000.

Fig. 37. A very young lateral thread group in section in the tangential wall of a sieve-tube just outside the cambium. There is no trace of any callus. The lower side is towards the cambium. × 1,000.

Fig. 38. A similar thread-group in a young radial wall. The pit membrane shows slight secondary pitting, and callus-heads have been formed at the ends of the threads on one side of the membrane. × 1,000.

Fig. 39. A tangential wall slightly older than Fig. 38. The callus has extended almost to the middle lamella from one margin of the membrane, and is just commencing on the other margin. The median dots have made their appearance at the lamella. × 1,000.

Fig. 40. A young sieve-field in section, somewhat overswollen on one side. One-half of the wall shows slime-strings in their callus-rods, and nodes at the lamella, whilst the other, younger half shows threads and heads of callus with the commencement of slime-strings. × 1,000.

Fig. 41. A sieve-field in the winter condition after treatment with Acid alcohol and Ammonia, and staining by the ordinary Safranin, Water-blue method. Only callus and median nodes are left. The callus-rods reach to the nodes, but do not touch each other. × 1,000.

Fig. 42. A field from the same material as Fig. 41 after treatment with 10 per cent. Potash for two days, and washing in weak Hydrochloric acid; callus and nodes have vanished and empty tubes are seen in the two portions of the split membrane. × 750.

Fig. 43. A transverse section of a sieve-tube after the action of Russow's Iodine solution and 75 per cent. Sulphuric acid. The walls have turned deep blue and have swollen greatly. Slime-strings and protoplasmic structures have vanished, and only the median nodes are seen as highly refractive bodies. × 1,000.

Fig. 44. A lateral wall of a sieve-tube in surface view. The sieve-tubes are composed of small groups of strings, and each group is covered by a head of callus formed by the union of the heads of the callus-rods which enclose each slime-string. × 750.

Fig. 45. A transverse section of the phloem, showing sieve-tubes and companion cells. One of the sieve-fields (*a*) in the radial wall has undergone further boring out to give a normal sieve-plate with thick slime-strings, and the field (*b*) shows an enlargement of some of its slime-strings. Small thread-groups are seen in the walls between companion cells and both sieve-tubes and bast parenchyma cells. × 1,000.

Fig. 46. A sieve-plate in section. The pit nearest the side-wall is furnished with strings of the sieve-field type. The others are ordinary sieve-plates. This sieve-plate belongs to one of the short sieve-tubes crossing a medullary ray. × 750.

Fig. 47. A piece of the phloem in tangential section, showing sieve-tubes with an inclined compound sieve-plate, a companion cell with its threads, bast parenchyma cells, and a group of threads between one such cell and a sieve-tube with the callus only on the sieve-tube side. Sieve-fields in surface view, &c. × 750.

Fig. 48. A piece of a radial wall of a sieve-tube in transverse section. The slime-strings of the sieve-field have been bored out to form definite continuous connexions between the two tubes. × 1,000.

Fig. 49. Wall between a bast parenchyma cell and a sieve-tube, showing groups of threads with callus-cushions on the sieve-tube side through which the threads are continued. × 750.

Fig. 50. A transverse section showing the connexions between two sieve-tubes and a bast parenchyma cell. The callus-rods reach as far as the middle lamella on the sieve-tube side only. The sieve-tube portion of the wall is the broader. Slime-strings are seen in the callus-rods and there are nodes at the lamella. Thread-groups between a companion cell and a sieve-tube are also seen, but callus is not indicated. × 1,000.

Fig. 51. A companion cell in longitudinal section. The threads in the walls between the cell and the sieve-tube do not appear to be homogeneous. On the cell side of the wall they are short and darkly staining, whilst on the sieve-tube side they are faint and longer and a trace of callus is seen on the surface. They are rather of the nature of fine slime-strings. A thread-group between the companion cell and a bast parenchyma cell is also shown. × 1,000.

Fig. 52. Wall between a companion cell and a sieve-tube in winter material. Callus is seen in the sieve-tube half of the wall. × 750.

Fig. 53. Young bast-fibres in transverse section, showing the funnel-like pits with groups of fine threads crossing the pit-closing membrane. A pit between a bast-fibre and a medullary-ray cell is also shown. Note the position of the lamella and the character of the pits. × 1,000.

Fig. 54. A compound sieve-plate in the winter condition in longitudinal section. The large callus-cushions are traversed by dotted lines, which are the remains of the slime-strings. Material preserved April 4, 1901. × 750.

Fig. 55. A sieve-plate in section, showing the recommencement of activity. Slime is beginning to accumulate in the sieve-tube and to bore its way along the old pores in the callus-cushions. Various stages are seen in the figure. Material preserved April 22, 1901. × 750.

Fig. 56. A callus-cushion in transverse section, showing the formation of new slime-strings in the old pores of the cushion on the reopening of the sieve-plates in spring. The strings are thicker than those seen in Fig. 55. Material preserved April 24, 1901. × 750.

Fig. 57. A sieve-plate in longitudinal section from material preserved May 9, 1901. The slime-strings are completely re-established and the callus is being rapidly dissolved. × 750.

Fig. 58. A callus-pad, in transverse section, which is undergoing solution. The pores are well seen and the whole cushion shows a stratified structure, which is revealed by the swelling due to the ferment action. × 700.

PLATE XVIII (Figs. 59–62).

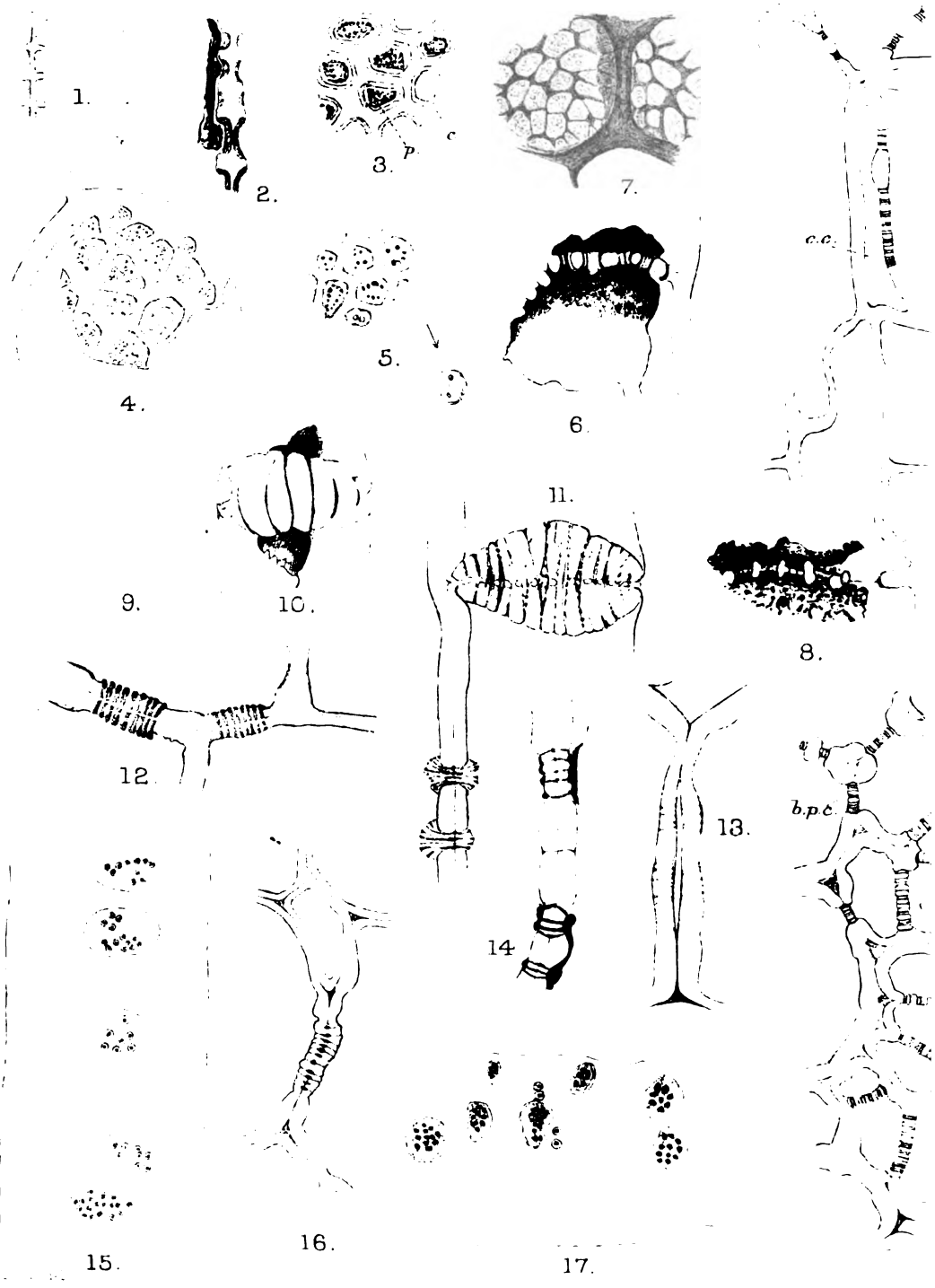
Tilia vulgaris.

Fig. 59. A sieve-tube in transverse section, showing the difference between the sieve-fields in the tangential and radial walls. In both cases they show median nodes, but in the tangential walls the connexions are very numerous and delicate. Callus is found in both cases, but is not indicated in the figure. × 1,000.

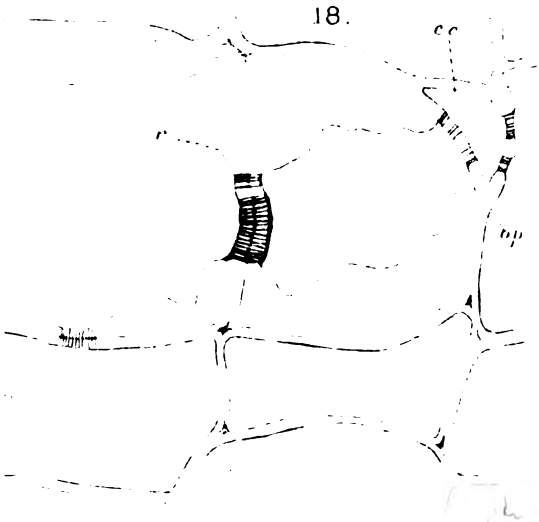
Fig. 60. A sieve-tube with two companion cells in transverse section. With Russow's callus-reagent, small callus-rods are seen in the sieve-tube half of the wall between companion cells and sieve-tubes. × 1,000.

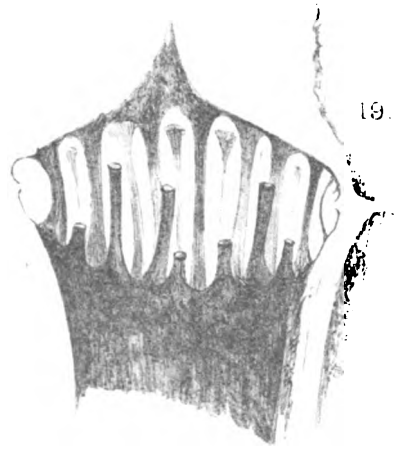
Fig. 61. A tangential wall between two sieve-tubes in surface view, showing the vast numbers of threads or fine slime-strings arranged in little groups. × 750.

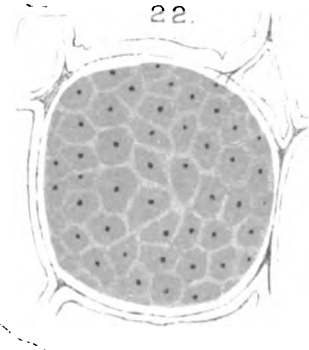
Fig. 62. A wall between a sieve-tube and a bast parenchyma cell in longitudinal section. A callus-pad is seen on the sieve-tube side over the groups of threads, but it could not be seen whether the callus extended to the lamella. Small median dots are seen on the threads. Winter material. × 1,000.

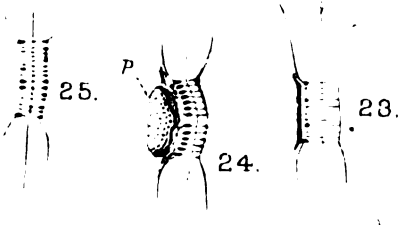


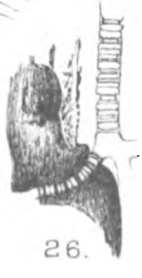
A.W. HILL. — SIEVE-TUBES OF ANGIOSPERMS.

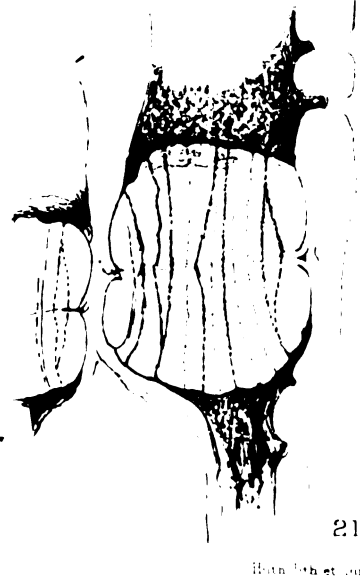












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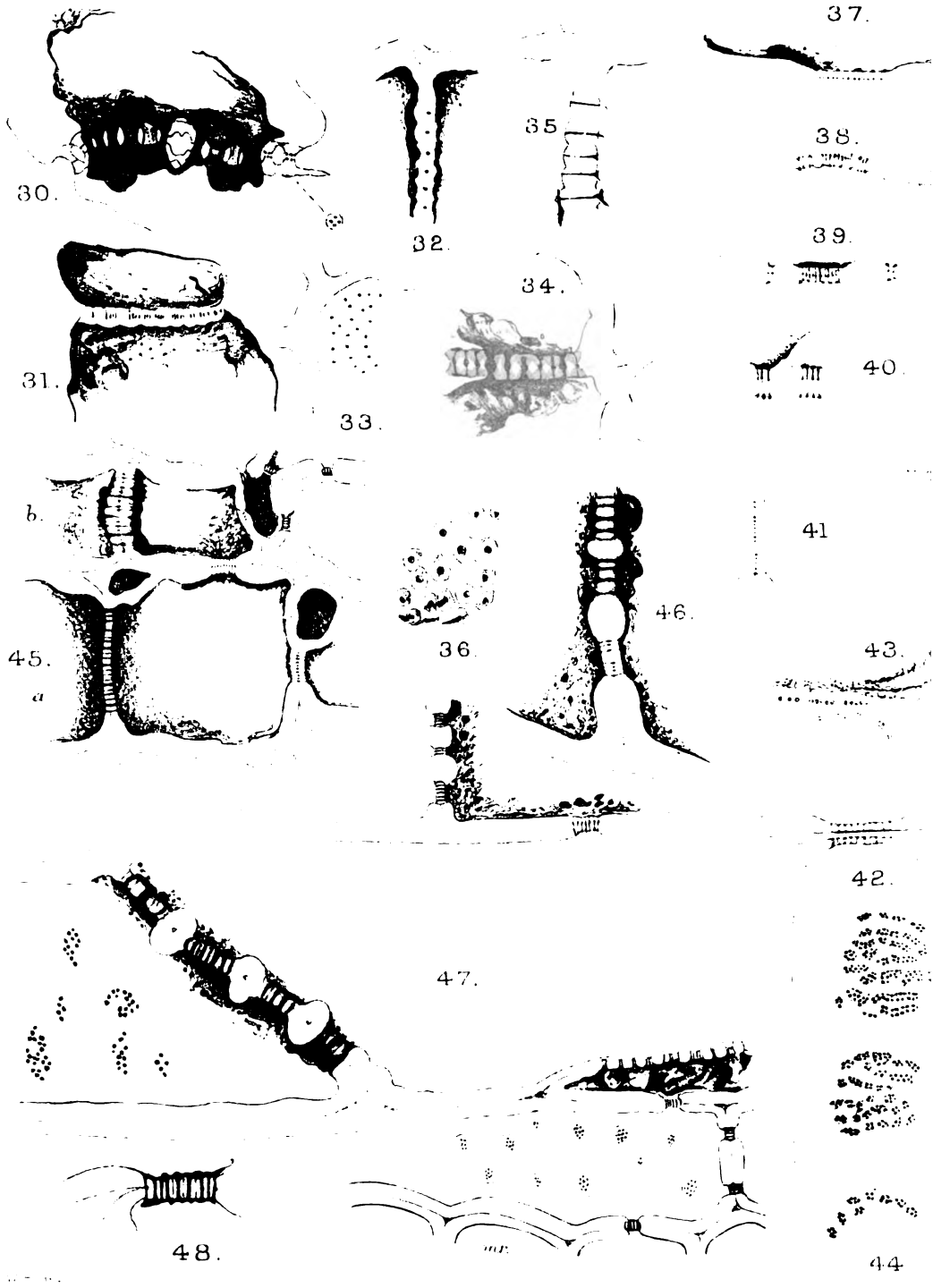

A.W. HILL — SIEVE TUBES OF ANGIOSPERMS.

Huth, lith. et imp.

Anatomy and Histology of Macrocystis pyrifera and Laminaria saccharina.

BY

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With Plates XIX, XX, and XXI.

AS much of the previous work on the anatomy of the Laminariaceae has led to contradictory conclusions, it appeared desirable that some further investigations should be made. Since only spirit or herbarium material has so far been examined, very little is known about the histology of these plants, and Mr. Hill therefore suggested that an examination of material preserved by more careful methods might yield useful results. I then undertook the study of some material of *Macrocystis pyrifera*, Ag. and *Laminaria saccharina*, Lamour., which had been previously collected and preserved by him, and I have since pickled and examined other material of *Laminaria sāccharina.*

I wish to express my sincere thanks to Mr. Hill for so liberally placing his material at my disposal, and also for much help and advice throughout the work. This research has been carried out at the Cambridge University Botany School.

I. Historical Summary.

(a) *Anatomy.*

The trumpet hyphae in the pith tissues of the Laminariaceae were originally discovered by Reinke [1], but Wille [2] in 1885 was the first to describe them accurately, and to suggest that they may possess a conducting function.

The larger sieve-tubes in *Macrocystis* had been noticed by J. J. Parker of Otago, but it remained for Will [3] to give a careful account of them with

[1] Reinke, Beiträge zur Kenntnis d. Tange. Prings. Jahr. f. w. Bot., 1876, Bd. x, p. 317.

[2] Wille, Siebhyphen bei den Algen, Ber. d. deutsch. bot. Ges., 1885; and Bidrag til Algernes Physiologiske Anatomi, Kongl. Svenska Vetens. Akad. Handl., B. xxi, No. 12, Stockholm, 1885.

[3] Will, Zur Anatomie von *Macrocystis luxurians.* Bot. Zeit., 1884.

figures both in *Macrocystis* and in *Nereocystis*. His description was followed by a paper from Rosenthal [1], who disagreed with him on many points concerning the anatomy and histology of *Macrocystis*. Three years earlier had appeared Oliver's [2] results, on the subject of the obliteration of the sieve-tubes in *Macrocystis* and *Nereocystis*.

In 1897 Macmillan [3] described the external features of *Nereocystis*, paying, however, only very small attention to the anatomy. Finally Wille [4], in 1897, again investigated the trumpet hyphae in *Alaria* and other Laminariaceae, and made an attempt to group the tissues in this family into various physiological systems.

Will [5] and Rosenthal [6] both studied the development of the young tissues in *Macrocystis*, and Setchell [7], during the course of his description of *Sacchoriza dermatodea*, gives a short and useful account of the youngest stages in that plant. A short summary of most of the above papers is given by Oltmanns [8]. The conclusions arrived at by these various authors are sometimes at variance, but the following is an attempt to sketch some of their principal points.

i. *Development in young plants.* The general ground-plan of structure in all Laminariaceae is the same. The tissues of the adult may be broadly differentiated into 'cortex' and 'central body'; though no sharp line can be drawn between these two regions, since it is found that in the course of development the inner layers of the cortex give rise to the outer layers of the central body. According to Setchell [7] the development of the central body in *Sacchoriza* takes place in the following manner. In the very young plant the innermost cells of the thallus soon cease to divide, and thus form the first constituent of the medulla, becoming much stretched owing to the great growth in length of the surrounding tissues. Later the adjoining layers of cells add to the medulla, and are, in a similar manner, stretched and elongated. The longitudinal walls of all the cells forming the central body become greatly swollen by the conversion of the middle lamella into slime, giving rise to the appearance of a number of isolated rows of filaments. These rows are connected by short cross connexions, which later become stretched by the swelling of the walls; they are considered by Setchell to be formed from the pit canals between the original central cells. In addition to these a large number of hyphae

[1] Rosenthal, Zur Kenntnis von *Macrocystis* und *Thalassiophyllum*. Flora, 1890, p. 105.

[2] Oliver, On the obliteration of the sieve-tubes in the Laminariaceae. Annals of Botany, i, 1887, p. 95.

[3] Macmillan, Observations on *Nereocystis*. Mem. Torrey Bot. Club, 1897, p. 273.

[4] Wille, Festskrift til H. Maj. Kong. Oskar II, 1897, Regieringsjubilant.

[5] Will, l. c., 1884. [6] Rosenthal, l. c., 1890.

[7] Setchell, W. A., Concerning the life-history of *Sacchoriza dermatodea*. Proc. Amer. Acad., 1891, 26, p. 177 (Crypt. Lab. of Harvard Univ., 17).

[8] Oltmanns, Morphologie und Biologie der Algen, vol. i, pp. 445-458.

grow in from the inner cortical layers and anastomose between the pith-tubes.

The descriptions given by Rosenthal[1] and Will[2] of young plants of *Macrocystis* bear a remarkable resemblance to Setchell's account of *Sacchoriza*, for in *Macrocystis* the original central cells also become stretched in the same way to form elongated filaments, and the number of these filaments is later added to from the inner 'cortical' cells. According to Rosenthal the formation of cross-connexions may take place in one of two ways. The first method is that noticed by Setchell in *Sacchoriza*, and consists in the pulling out of a pit canal, owing to the swelling of the longitudinal walls of the pith elements, and in the formation of cross walls in the canal thus produced. The second method, which is, he says, more common, begins by the formation of a lateral protuberance which grows out from the longitudinal wall of one cell, forming a true hypha-like outgrowth, and, after a shorter or longer period of wandering, makes a secondary connexion with some other cell, the two cells connected being either quite near together or at some distance from one another.

A large number of hyphae also grow in from the inner cortical cells, and, wandering in the substance of the swollen middle lamellae of the cells of the medulla, are responsible for much of the later growth of that region. Setchell states that in *Sacchoriza* broken cross connexions can grow out and form hyphae, and he also thinks that some of the hyphae formed from the inner cortex may assume a longitudinal course in the pith, and hence resemble, except for their smaller diameter, the original pith filaments. From the various accounts it is uncertain whether the ingrowing hyphae ever form secondary connexions in the medulla. Rosenthal[3] and Oltmanns[4] assert that they never do so, but always have free endings, and on the whole this appears to be the opinion of most authors except Will[5] and Wille[6], who believe that new connexions may sometimes arise.

ii. *Growth in thickness.* The method of increase in diameter in the stem of *Macrocystis* has been a difficult question to solve. It is known that in the Laminariaceae growth in thickness is entirely due to the outer layers of the cortex, which divide rapidly by tangential and radial divisions. The same kind of meristematic growth certainly does take place in *Macrocystis*, and it is principally to this method of cell division that Rosenthal ascribes the increase in thickness in that plant. He points out that, while in the young stem the width of the cortex is approximately equal to that of the pith, in the old stem it may be ten times as great. On the other hand there can be no doubt that the diameter of the pith in the old plant is much greater than in the young one, but as to the method by which it has been

[1] Rosenthal, l. c., 1890.
[2] Will, l. c., 1884.
[3] Rosenthal, l. c., 1890.
[4] Oltmanns, l. c.
[5] Will, l. c., 1884.
[6] Wille, l. c., 1885 and 1897.

increased there is some difference of opinion. Rosenthal considers that its growth is to be wholly ascribed to the slow addition of new elements on its periphery by transformation from the inner cortex, the only meristematic layer being the outer layer of the cortex. He lays great stress on the distinct radial rows to be traced, in the young plant, from this outer layer right into the centre of the pith. He supposes the outer cortex to be continually added to on its outer edge, and transformed slowly into inner cortex on its inner edge. The inner cortex differs from the outer in being composed of more elongated cells, and it is these which, in their turn, give rise to those peculiar elements found only in *Macrocystis* and *Nereocystis*, which are usually regarded as true sieve-tubes, and which are found, in all except the very young stages, arranged in radial rows at the periphery of the pith.

In a stage slightly older than that in which sieve-tube formation begins, when most of the original inner cortex has been already used up in their production, the remainder of that tissue presents a rather striking appearance, and it is to this narrow band that we will return below when discussing Will's theory of secondary growth in *Macrocystis*.

The later formed inner cortex is made up of cells much less elongated than are those of the earlier layers, and hence there is a more or less sharp distinction between the two tissues. No more sieve-tubes are formed after a certain stage (probably, according to Rosenthal, when all the original inner cortex has been used up), and the whole of the further growth in thickness of the medulla is due to the expansion of the already formed tissues, and to the ingrowth of cortical hyphae.

The alternative view, due to Will, is that the production of new sieve-tubes may go on indefinitely, and Oliver[1] appears to have accepted his theory. Will attributes the formation of these elements, in the young plant, to a special 'thickening ring' which corresponds both in position and appearance with the narrow band of tissue referred to above, and which he believes functions as a separate meristem.

Will states that in the older plant this 'thickening ring' has no definite boundary, but a gradual transition between inner cortex and sieve-tubes is then found. It is not at all clear from his account whether he means to imply by this statement that there is only an apparent transition, or whether he wishes to draw the inference that actual transition from inner cortical cell to sieve-tube is at the moment taking place. One would suppose, however, from the general terms of his description, that, unlike Rosenthal, he considers growth in thickness, by means of a secondary formation of new sieve-tubes, to be a long continued process, and that he therefore believes in the production of new young sieve-tubes in the old plant.

[1] Oliver, l. c., 1887.

Neither does Oltmanns'[1] account of the production of the sieve-tubes in *Macrocystis* help to clear up the difficulty.

No definite secondary growth in thickness has ever been described in any other Laminariaceae except *Nereocystis* and *Thalassiophyllum*. The former appears to agree closely with *Macrocystis*[2], both in its mode of development and in its adult anatomy, but the latter, described by Rosenthal[3], has an inner cortical meristem which takes the place of the original outer cortical meristem, the latter soon ceasing to function.

iii. *Morphological nature of the various elements composing the medulla in the Laminariaceae.* The terminology of the various elements composing the medulla has been a matter of some difficulty.

(α) It is fairly agreed that the large outer tubes in *Macrocystis* and *Nereocystis* are probably true sieve-tubes (Oltmanns, Oliver, Will, Wille, Rosenthal), but it is uncertain whether the original pith cells should be included in this category. These have been termed 'trumpet hyphae' by Oliver, but Oltmanns[4] has recently pointed out that, whether they are to be looked upon as sieve-tubes or not, they are certainly not hyphae. The latter suggests that probably both they and the large sieve-tubes will be found to possess sieve-plates, whose manner of formation will prove to be similar to that of the true sieve-plates in the Phanerogams.

Wille[5] also considers the 'sieve-cells' or central pith cells, both of *Laminaria* and of *Macrocystis*, to be homologous with the larger sieve-tubes of *Macrocystis*; whilst Oliver, on the other hand, maintains that they are of an entirely different nature, and are not to be regarded as true sieve-tubes. He lays stress on the fact that he found no cross connexions in *Macrocystis* between the pith-cells, or 'trumpet hyphae', and the true sieve-tubes.

Oliver[6] and Wille[7] both bring forward the suggestion that the presence of these large sieve-tubes in *Macrocystis* and *Nereocystis* is correlated with the habit of these plants.

Oltmanns, however, suggests that their function may be in part mechanical.

(β) *Hyphae.* Various elements in the medulla have been termed hyphae by different authors, but it seems generally agreed that this loose use of the term is not to be commended, and that only the ingrowing filaments, arising from the cortex, can be really looked upon as true hyphae. It is as yet uncertain whether these form secondary connexions.

iv. *Mucilage canals.* The mucilage canals in *Macrocystis* and other Laminariaceae have been carefully described and figured by Will[8] and others, and do not immediately concern this account.

[1] Oltmanns, l. c., p. 452.
[2] Macmillan, l. c., 1897. Oliver, l. c., 1887.
[3] Rosenthal, l. c., 1890.
[4] Oltmanns, l. c., pp. 453-454.
[5] Wille, l. c., 1897.
[6] Oliver, l. c., 1887, p. 112.
[7] Wille, l. c., 1897.
[8] Will, l. c., 1884.

(b) *Histology.*

Owing to difficulties of technique very little histological work has been done on properly preserved material of the brown algae.

i. *General cell-wall.* The mucilaginous nature of the general walls has been noted by several authors and has been contrasted with the harder substance which forms the pit-closing membranes and the sieve-plates. The middle lamella is said to be largely composed of the calcium salt of tungstic acid, which is analogous to the calcium pectate of higher plants. Rosenthal[1] gives some account of the distribution of the pits in the cortex of *Macrocystis*, where they are confined chiefly to the end walls, but are also present occasionally on the lateral walls; protoplasmic threads have not been figured.

ii. *Development of the sieve-plate in Macrocystis.* In connexion with Rosenthal's hypothesis of the formation of sieve-tubes from the cortex of that plant, he brings forward a remarkable theory to account for the origin of a sieve-plate from the cross wall of a cortical cell. The latter has usually a single ring of pits in its end wall, but towards the pith Rosenthal says that cross walls are found in which the inner edges of these pits become indistinct, and finally the intervening wall in the centre of the ring is partly dissolved. Only a thin membrane is then left across the cell, having still a thick edge which represents the unaltered part of the wall outside the ring of pits. The thin membrane becomes the sieve-plate, and is finally found to be perforated by numerous holes, which are larger in the middle and smaller towards the periphery; the origin of these holes appears to be somewhat obscure. Rosenthal found but few lateral plates on the walls of the sieve-tubes, and notes that the lateral pits of the cortical cells would naturally become much separated after the latter had given rise to sieve-tubes by a stretching process.

Oliver[2] describes the formation and obliteration of the pores of the sieve-plate in *Macrocystis* and *Nereocystis*. He describes and figures protoplasmic threads in the cross walls of the young sieve-tubes, and also gives some details of the formation of callus and slime-strings in the sieve-plates; his paper, however, is chiefly concerned with the older stages in which callus has already begun to accumulate. He considers the callus masses which are formed on either side of the sieve-plates, and the long strings of callus which often entirely fill the lumen in the old trumpet hyphae, to be formations from the cell-wall, and he figures cases where the callus-stain is seen to fade into the wall, showing the change to be a gradual one.

[1] Rosenthal, l. c., 1890.
[2] Oliver, l. c., 1887, Figs. 89, &c., Pl. VIII.

Callus and slime-string formation are also described by Will [1], Wille [2], and Rosenthal [3] in *Macrocystis*, and by Wille [2] in *Alaria*, and the suggestion is several times advanced that the development of the sieve-plate will be found, on further investigation, to be similar to that in Angiosperms. Since all these authors worked on dried or alcoholic material, the finer histological details of the protoplasmic threads and of callus-formation could not be studied.

iii. *Protoplasmic continuity.* Hick [4] in 1885 attempted by various and somewhat drastic methods to demonstrate protoplasmic continuity in *Ascophyllum* and *Fucus*. The chief interest of his experiments lies in the demonstration which they afford of the great resistance of these algal cell-walls to powerful chemical reagents.

Rosenthal's attempts to demonstrate continuity of protoplasm appear to have been more successful. He does not figure his results, but describes slime-strings in the sieve-tubes and connexions through the pit-closing membranes of the cortical cells.

Oliver also describes protoplasmic continuity in the young trumpet hyphae and sieve-tubes of *Macrocystis*.

(c) *The objects of the present research.*

From the foregoing review it will be seen that several points require investigation.

I. *Anatomical.*

i. The morphological nature of the 'trumpet hyphae' and so-called 'true sieve-tubes' in *Macrocystis* and *Laminaria*.

ii. The nature of the growth in thickness of the medulla in *Macrocystis*.

iii. The formation of secondary connexions by the hyphae growing into the medulla in both plants.

II. *Histological.*

i. The question of the presence or absence of protoplasmic connecting-threads across the cell-wall.

ii. The development of the sieve-plate in *Macrocystis* and *Nereocystis*, and of the cross walls of the trumpet hyphae in these and other species, and its comparison with the development of the Angiospermous sieve-plate [5].

iii. The nature of the callus found obliterating the older sieve-plates and formed throughout the length of the trumpet hyphae and old sieve-tubes. The question whether it is a cell-wall production, due to alteration of already formed cellulose, as described by Oliver, or whether it is deposited by the

[1] Will, l. c., 1884. [2] Wille, l. c., 1885. [3] Rosenthal, l. c., 1890.

[4] Hick, Protoplasmic Continuity in the Fucaceae. Journal of Botany, xxiii, 1885.

[5] Hill, A. W., Histology of the sieve-tubes in *Pinus*. Ann. of Bot., xv, 1901. Historical Summary, pp. 576–585.

protoplasm in the same way as cellulose, as has been suggested by Russow [1] and Strasburger [2], and found by Hill [3] in *Pinus*.

II. Material and Methods used in this Investigation.

Material. The material of *Macrocystis* which was at my disposal consisted of the young apex of a growing plant, and of two collections of older portions of the stem. One of the two collections was made in the Falkland Islands, and consisted of pieces of stem of about one-third of an inch in diameter, while the other came from Talcahuano Harbour, Chili, and was obtained from rather younger plants whose stems varied from one-eighth to a quarter of an inch in diameter. The young apex was preserved in spirit, and the rest of the material was fixed in a solution of Iodine in Potassium Iodide, dissolved in sea-water, and preserved in a Thymol solution. All the material was obtained by Mr. Hill in December (1902), that is, early in the South American summer.

I had also the opportunity of examining all stages in the development of young plants of *Laminaria saccharina*, preserved in spirit. These young plants were used in investigating anatomical development, while older material, preserved in September by Mr. Hill, and in February by myself, had been treated in a manner which rendered possible more minute histological work. Some of the September material was fixed in a solution of Iodine in Potassium Iodide, and the rest in Picric acid, both being preserved in Thymol; but neither of these methods was found to be very successful. Various methods of fixation at different temperatures were tried as experiments on the February material.[4]

Methods. The methods used in swelling and staining were based on those already published by Gardiner [5] and employed by Hill [6] in *Pinus*. A solution of Iodine in 10 per cent. Potassium Iodide was found to be the most useful swelling agent, and gave good results when the material was left in the solution from two to three weeks.

For staining, a modification of the Safranin method [7] was used in conjunction with Aniline blue [8] and London blue. The chief difficulty met

[1] Russow, Sitzb. der Dorpater Nat. Ges., 1882; Ann. des Sc. Nat., 6e sér., t. xiv, 1882.

[2] Strasburger, Bot. Pract., 1884.

[3] Hill, l. c., p. 598. For a summary of the various views on the origin of callus, see pp. 597–600.

[4] Some of the fixing agents used at the suggestion of Mr. Hill were Picric acid, Iodine in Potassium Iodide, Picro-Acetic acid, and Picro-Uranium Nitrate, all being dissolved in sea-water, and Thymol being used in all cases as a preservative.

Picro-Uranium Nitrate was found to give the best results, the composition of the solution used being as follows:

50 cc. sea-water.
·5 gr. Picric acid.
1 gr. Uranium Nitrate.

[5] Gardiner, Proc. Camb. Phil. Soc., 1898; Proc. Roy. Soc., vol. lxii, 1897.

[6] Hill, I. Connecting-threads in *Pinus* (Pt. 1 of Gardiner and Hill on the Histology of the Cell-wall), Phil. Trans. Roy. Soc., Bot., 1901, pp. 83–125. II. Histology of Sieve-tubes in *Pinus*, Annals of Botany, vol. xv, 1901, p. 576.

[7] Gardiner, l. c., 1898, p 508.

[8] Water blue made up with aniline.

with was the impossibility of removing the Safranin from the walls by any of the ordinary methods, but a solution of dilute glycerine followed by London blue was found to produce the desired result. The London blue not only removes the Safranin from the walls, but also stains the callus, as does Water blue. Moreover it has the additional property of staining callus in places where Water blue has no effect, presumably because the callus is not sufficiently hydrated.

The acid-violet method [1] both by itself and in conjunction with Safranin was found to be useful, and Water blue made up with Aniline was occasionally employed alone.

London blue alone was used in the investigation of callus, and Methylene blue, Thionin, and Fuchsin were found to be convenient cell-wall stains.

III. Macrocystis pyrifera.

A. *General Anatomy.*

As no young plants of *Macrocystis* were available, my investigations of the development were confined to the study of the apical portion of an adult specimen.

Stage i. In the youngest part of the growing thallus the tissues are already differentiated into cortex and medulla, the breadth of the cortex being somewhat greater than that of the medulla.

The outermost layer of the cortex is composed of regularly arranged isodiametric cells, which are in a state of active division, and give rise to rows of elements, the radial arrangement of which can be traced in transverse section throughout the cortex, and often appears to be continued into the medulla. About eighteen layers of cells make up the entire cortex, the counting being made along any such radial row. The outer eight or ten layers are certainly of secondary origin. In longitudinal section they are seen to be composed of thin-walled parenchymatous elements, sparsely provided with contents, and on the extreme periphery of the section mucilage canals with small patches of secretory cells (cf. Will [2]) are present in abundance.

In longitudinal section this outer cortex is sharply marked off from the inner, which is made up of much more elongated cells, whose longitudinal walls are considerably swollen, while many of their transverse walls are very thin and give the impression of having been quite recently formed. It seems certain that this inner cortex represents the primary cortical tissue of the thallus, the cells of which have given rise to these characteristic elements by means of longitudinal stretching followed by rapidly succeeding cross

[1] Gardiner, l. c., 1898, p. 508.

[2] Will, l. c., 1884.

divisions. The cells of this inner cortex have very dense contents and large nuclei. Pits are present in their thin transverse walls, but they are not very easy to see, being more numerous, smaller and shallower than in any of the other walls. They do not appear, either in the inner or outer cortex, to be arranged in a ring as are the pits in the transverse walls of the cortical cells in older material. A few pits are also present in the longitudinal walls.

The innermost layers of primary cortex give rise to hyphae which run into the medulla. The free ends of these hyphae were never certainly seen, and it seems probable that secondary connexions are formed among them, but to find definite proof of this it would be necessary to study their development in young plants.

The medulla at this stage is nearly circular in cross section, and is composed of elements which are still more elongated than those making up the inner cortex, and have also more swollen walls. Between these elements, and in the mucilaginous substance formed from the swollen middle lamella of their walls, run numerous, much branched hyphae. In a transverse section at this stage there is no definite boundary line between the medulla and inner cortex, and the elements of the medulla themselves do not appear to be sharply differentiated as to size or other characters; but in longitudinal section it is seen that those forming the one or two outer rows pursue a considerably straighter course than the others, and are also somewhat wider, having more dense contents and larger nuclei. The elements composing these outer rows are the first of the true sieve-tubes, but at this stage are quite immature.

The central region of the pith or medulla is occupied by numerous sieve-tube-like elements, the 'trumpet hyphae' of Oliver. These are presumably the original central cells of the thallus which have become stretched by the growth of the outer tissues and separated by the hyphae growing in from the cortex and anastomosing between them, but never forming connexions with them. These central elements, which it will be useful to call the 'primary pith filaments', though they do not pursue a very straight course, are yet only seldom branched, except indeed at the origin of a leaf segment. At such a point it is usual to find numerous examples both of pith filaments and sieve-tubes dividing into two, one branch continuing its course in the main axis, while the other turns off into the leaf. Callus is apparently formed very early in the central part of the medulla, and even at this stage it is not unusual to find long strings of callus in course of formation. It seems probable, however, that in a slightly younger stage these elements must have functioned for conduction, but that, after the development of the sieve-tubes from the inner cortex, they have acquired a mechanical function, since their lumina have become obliterated by callus.

Stage ii. Commencement of rapid growth in thickness. In a stage slightly older than the one described above, the medulla has become oval in outline, and sieve-tubes are being rapidly produced by the stretching of the inner cortical cells, while the outer cortex is considerably enlarged by the divisions of the external meristem.

The inner cortex is still sharply marked off from the outer, while between the inner cortex and the medulla there is no clear line of distinction.

Every stage of transition between sieve-tube and cortical cell is present. The sieve-tubes on the periphery of the medulla consist of short elements hardly swollen at the cross walls, while the innermost ones are composed of elongated cells much dilated at the cross walls. Branching of the young sieve-tubes is fairly common, and cross connexions, developed from the pit canals by the stretching of the pits due to the swelling of the longitudinal cell-walls, are very easily traced at this level in their various stages of development. There is a considerable amount of callus in the primary pith filaments, but even in the innermost sieve-tubes callus formation has hardly commenced.

Stage iii. Sections taken about one and a half inches below the apex show a much more advanced stage of development. (Fig. 44, Pl. XXI.)

The cortex is now about four times the breadth of the medulla, and is no longer sharply differentiated into regions, but there appears to be a gradual transition, from the outer parenchymatous to the more elongated inner elements; the latter give rise to numerous hyphae. The whole of the characteristic inner or primary cortex described above [1] has been transformed into sieve-tubes, and all the cortex now present is of secondary origin, derived from the repeated divisions of the outer cortical meristem.

Young sieve-tubes are still present on the outer edge of the medulla, but it seems improbable that new ones are being formed at this stage. The zone of sieve-tubes now contains from seven to nine elements in each radial row, and the development of the sieve-plates is rapidly proceeding, callus being already present in the innermost rows. The 'primary pith filaments' all contain callus.

Stage iv. In a stem of about twice the thickness of that last described but little change appears to have taken place.

The almost isodiametric cells of the outer cortex have now deeply pitted walls, and the pits are distributed fairly evenly over the longitudinal, and arranged in a ring in the transverse, walls. In the more elongated and thicker-walled cells of the inner secondary cortex the pits are also arranged in a ring in the transverse walls, but in the longitudinal walls they are very few and isolated. The appearance of these latter cells, as well as the

[1] pp. 297–298.

arrangement of the pits in their walls, suggest that their function may be the conduction of water.

A much more definite boundary can be recognized between the cortex and medulla at this stage, at least in a transverse section, but this appearance is partly due to the fact that the ingrowing hyphae now originate only from the innermost cortical layer. From this layer they arise in large numbers and run more or less radially towards the centre; they often branch, and are thus to a large extent responsible for the increase in size of the medulla.

From six to nine sieve-tubes are present in each radial row, and the innermost element of each row is generally already quite filled with callus. The primary pith filaments are more widely separated than in the younger stages, and in many cases their lumina are choked by callus.

Stage v. The oldest stem which I examined was about half an inch in diameter (cf. Fig. 45, Pl. XXI).

From this stage nothing is gained to add to the description of the cortex already given in the last stage, beyond the fact that it is now eight or nine times broader than the medulla.

Not more than nine rows of sieve-tubes, the two or three innermost rows of which are now completely obliterated by callus, were found in any radial row; it would seem probable, therefore, that no new ones have been formed since the original primary inner cortex was transformed to the sieve-tube layer. On the other hand, young sieve-tubes composed of quite short cells without callus are still present, and in longitudinal section are often difficult to distinguish from the innermost cortical cells, though in cross section they are sharply differentiated from the latter by the much greater thickness of their walls. The innermost cortical cells have always a ring of pits on their transverse walls, while the young sieve-tubes have definite sieve-plates, and it is not easy to imagine how an adult wall with its ring of pits (such as that figured in Figs. 1 and 2, Pl. XIX) could give rise to a sieve-plate. Certainly no evidence of such a transition is forthcoming. In order to prove definitely that young sieve-tubes do not continue to be formed slowly from the inner secondary cortex throughout the life of the plant, it would be necessary to investigate some considerably older material. On the whole, however, from the evidence forthcoming, it seems probable that the increase in size of the medulla in the oldest specimens is due partly to the great increase in diameter of the elements composing it, but still more to the large number and repeated branching of the hyphae anastomosing among them.

In this old material hyphae which contain callus are occasionally met with. Some of these have taken a longitudinal course, and have very much the appearance of extremely narrow sieve-tubes. They are composed of elongated cells, swollen at the cross walls, and have dense contents;

there can be little doubt that Setchell[1] is referring to elements of this nature when he describes the transformation of some of the anastomosing hyphae of *Sacchoriza* into conducting-tubes.

B. *Histology.*

i. *Nature of cell-walls.*

The mucilaginous nature of the cell-walls of both cortex and medulla in *Macrocystis* is well shown by their great affinity for Methylene blue. In this and other respects, however, both the pit-closing membranes of the cortical pits, and the sieve-plates, show a marked difference in reaction from the rest of the walls. The pit-closing membranes do not stain at all with Methylene blue, but the sieve-plates, with the exception of the small areas actually traversed by threads and slime-strings, take up the stain after remaining in it some hours; cf. Fig. 1, Pl. XIX.

With Congo red the outer layers of the cell-wall stain pink, but the middle lamella, pit-closing membranes, and sieve-plates remain unstained.

The cell-walls of both cortex and medulla give the ordinary cellulose reaction with Iodine and Sulphuric acid, but the middle lamella is very resistant, and remains yellow for a long time before it turns blue, whilst the actual membranes of the pits and the sieve-plates remain permanently yellow.

In their response to swelling agents, it is found that the pit-closing membranes and sieve-plates differ from the cell-wall. Picro-sulphuric and other acids cause the latter to swell considerably, but produce little or no result on either of the former. A solution of Iodine in Potassium Iodide, on the other hand, has not so marked an effect on the cell-wall, while prolonged treatment with this reagent finally causes the pit-closing membranes and sieve-plates to swell slightly. No swelling agent yet used has been able to overcome their powers of resistance to any great extent.

From the above it would appear that the chemical nature of the cell-wall is of the following constitution. The middle lamella is chiefly composed of pectic compounds with some cellulose, but in the outer layers of the wall the proportion of cellulose to pectose is increased. The pit-closing membranes and sieve-plates are evidently composed of some substance still more resistant than pectose.

ii. *Protoplasmic continuity in cortex and hyphae.*

a. Cortex. The demonstration of protoplasmic connecting-threads in the cortical cells of *Macrocystis* is, owing to the resistant nature of the pit-closing membrane, a matter of some difficulty, but satisfactory results were obtained by means of an adaptation of the Safranin method.

[1] Setchell, l. c., 1891.

In surface view (Fig. 2, Pl. XIX) a few threads, not more than six or seven, are seen in each cortical pit, and in longitudinal section (Figs. 3 and 4, Pl. XIX) these threads may be seen as very short lines which cross the pit-closing membrane and unite the protoplasm of adjoining cells. These connecting-threads were seen in old plants in the pit-closing membranes in the transverse and longitudinal walls of both inner and outer cortical cells.

b. Hyphae. Numerous single fine threads were demonstrated in the cross walls of the anastomosing hyphae in the young material (Fig. 24, Pl. XIX), but in the older material they are not visible, and it seems probable that the great swelling of the walls has stretched and broken them. It is impossible to be certain which of the cross walls are due to secondary fusions among the hyphae, and one cannot, therefore, say whether threads are developed at places where secondary fusions have occurred. To clear up this point an examination of young plants will be necessary.

iii. *Development of the sieve-plates and obliteration of the sieve-tubes.*

a. Primary pith filaments. Owing to the absence of sufficiently young material the development of the cross walls of the primary pith filaments could not be followed, since callus was already present in nearly every case investigated. In one instance (Fig. 26, Pl. XIX) knobs were seen on either side of the cross wall, but it was impossible to be certain that they were connected by threads, as this stage was observed in spirit material.

Callus is very soon formed, and Fig. 28, Pl. XIX, represents a very commonly occurring early stage, in which callus-rods are found traversing the sieve-plates and a callus-pad has also been already laid down on either side of the plate. There seems no doubt that the mass of callus shown here and in the later figures is laid down by the protoplasm, and is not formed by an alteration of the wall already present, for the original wall, undiminished in thickness, can still be seen, and it does not seem possible that such a large amount of callus could be produced merely by the swelling of an immeasurably small outer layer of this wall.

Fig. 29, Pl. XIX, represents a case in which the callus is accumulating in the centre of the plate, while Figs. 30, 31, Pl. XIX, are drawn from older stages in which a larger amount of callus has been formed. The callus usually accumulates much faster on the side farther from the apex. In Fig. 31, Pl. XIX, the protoplasm has begun to lay down callus throughout the length of the tube. It is formed first as a thin line on the edge of the protoplasm, but soon becomes irregular in its distribution, being laid down in small wedges or blocks, adjoining especially dense portions of protoplasm. These wedges (cf. Fig. 23, Pl. XIX) meet across the tube in places and nearly choke up the channel, but for some time a thin layer of protoplasm is left lining

the interior of the tube. Later, this also is often obliterated and the cavity becomes entirely blocked.

b. Secondary sieve-tubes. (i.) *Surface views.* A study of the earliest stages in the development of the terminal sieve-plates, both in the young material and in the sieve-tubes found on the periphery of the medulla in the older stems, yields very similar results. In the youngest sieve-plates there is a uniform distribution of the threads. Fig. 5, Pl. XIX, represents a slightly older and very much more frequent case in which the centre of the plate is still occupied by single threads, but towards the edge little groups of four or five threads are present; between the centre and the periphery groups are seen composed of two or three threads. Other cases are found in which the centre of the plate is in this last stage, while groups of four to seven threads are present on the periphery. In Fig. 6, Pl. XIX, is shown an older plate, just before callus formation, in which the threads are distributed throughout in groups, five being the usual number, though sometimes as few as four or as many as seven threads may be present in one group. These stages were seen in preparations stained with Safranin, Safranin and Aniline blue, Aniline blue only, and Benzyl blue, so that there seems little chance that any of them can be an artificial product.

Numerous estimations were made of the numbers, both of the separate threads in the youngest sieve-plates and of the groups of threads in the later stages, and there is no doubt that the two quantities broadly correspond. It seems impossible, therefore, that the groups are formed by the aggregation of the original single threads; rather, it seems likely that each group has arisen from the division of a single thread. This conclusion is also supported by the fact that groups of two or three threads are often found in an intermediate position between the earliest arrangement of separate threads and the final resulting groups of five or more.

In a surface view of a sieve-plate callus is first seen as a small ring surrounding each of the threads which make up such a group, and a young sieve-plate in this stage shows numerous small circles each made up of four to six blue points, as shown in Figs. 8 and 9 *b*, Pl. XIX. By lateral fusion this group of separate spots soon gives rise to a single ring (Figs. 8 and 9 *c*, Pl. XIX).

All this time the threads remain distinct, though some change takes place in their composition, for, simultaneously with the advent of the callus they stain more deeply than before with protoplasmic dyes.[1] Soon after the fusion of the separate callus-spots into a single ring, alterations take place very rapidly at the centre of each group of threads. The final result is that a single slime-string is formed, surrounded by its ring of callus, in place of the original group of independent threads (Fig. 7 and Figs. 8 and 9 *c*, Pl. XIX). Before arriving at this stage two or three smaller slime-strings are often

[1] Cf. Hill, l. c., 1901, II, p. 589.

seen (Figs. 8 and 9 *d*, Pl. XIX) situated in a blue area which represents the original position of a group of threads, and these, together with the intervening callus and wall, when any unaltered wall is still present, appear to fuse and disorganize, giving rise to the single slime-string.

The sieve-plate is now fully developed and is a distinct sieve with open pores, through which, in optical section, slime-strings are often seen to run, though in many cases, owing to the thinness of the sections, they have fallen out of the pores during the processes involved in staining the preparation. Three to five sieve-tubes with sieve-plates in this condition may be found in any radial row in the oldest material of *Macrocystis* examined.

The obliteration of the innermost active sieve-tubes appears to be a very rapid process. Callus spreads over the surface of the sieve-plate in between the threads and then begins to accumulate at the centre of the plate. The mucilaginous and protoplasmic contents of the tube are at this stage collected into a densely staining mass over the central pad of callus (Fig. 17, Pl. XIX) and continue to deposit more callus, gradually increasing the size of the pad. A thick layer is thus spread over the whole plate and a callus-mass results (Fig. 19, Pl. XIX) on both sides of the plate, but is usually larger on one side than on the other. Meanwhile callus is often formed down the whole length of the tube (Figs. 20, 23, Pl. XIX), in a manner similar to that described above [1] for the primary pith filaments, and accumulates in small masses which are laid down by the dense protoplasm lying against the wall of the tube, till finally in many places the lumen is almost obliterated (Fig. 21, Pl. XIX).

ii. *Longitudinal sections of the terminal sieve-plates.* In longitudinal sections the course of events is not always easily followed. In the youngest sieve-plates examined, the threads are very delicate and often much broken, but they can be seen to be arranged singly (Fig. 11, Pl. XIX). More difficulty was experienced in following the formation of the groups of threads, as the sieve-plate at this stage often becomes very swollen. In several cases single threads were seen in the central portion of the plate, while groups occurred on the periphery. Fig. 13, Pl. XIX, shows the occasional appearance of groups of threads in a plate in which the separate arrangement is still prevalent. In numerous sieve-plates which as yet had no callus, the threads could be clearly seen to be arranged in groups throughout. In several cases a median node, such as that described in *Pinus* [2], was seen on each thread, and in one such instance callus had been formed and the threads were still visible traversing the callus-rod.

With the appearance of the callus the threads stain much more deeply with Safranin. These alterations are first noticed at the periphery of the

[1] p. 302.

[2] Hill, The histology of the sieve-tubes in *Pinus*. Ann. of Botany, xv, 1901, p. 589, and Figs. 8, 10, &c., Pl. XXXII; and p. 590 and Fig. 20, Pl. XXXIII.

plate and then proceed towards the centre, the periphery being therefore generally more advanced in development than the centre; they also always begin at the ends of each of the threads of a group and work towards the middle lamella. Sometimes it was found, as in Figs. 14 and 15, Pl. XIX, that callus formation had begun on one side only of the plate in any given group, and had gone as far as the middle lamella from that side, while on the other side unaltered threads were present, as yet not even changed with regard to their staining properties.[1]

In many cases each thread can be clearly seen to have its own callus-rod, but the separate callus-rods soon fuse, and a single rod is formed, through which the deeply staining threads are seen to pass. These threads then disorganize and, together with the intervening callus, give rise to a single slime-string. Numerous preparations, such as those shown in Figs. 15, 16, Pl. XIX, were obtained in which callus and slime-string production had proceeded a certain distance from each side of the sieve-plate, and unaltered threads, or threads each enclosed in a separate callus-rod, could be seen stretching between the cones thus formed. In some sieve-plates (Fig. 16, Pl. XIX) this stage of development had been reached on the periphery, while in the centre groups of unaltered threads are still present. The middle lamella then becomes dissolved, at the point at which two cones finally meet, and a continuous slime-string enveloped in a tube of callus is thus obtained; the perforation of the sieve-plate is now complete.

The stages in the obliteration of the innermost sieve-tubes are more clearly seen in longitudinal than in transverse sections. In Fig. 16, Pl. XIX, callus formation can be seen to have spread, from the heads of the rods surrounding the slime-strings, over the intervening areas. In Fig. 17, Pl. XIX, a small pad of callus has been formed over the central portion of the sieve-plate, and in Fig. 19, Pl. XIX, such a stage as this has given rise to a large mass, extending right across the sieve-plate, and filling up a large portion of the sieve-tube. From this it seems clear that the obliteration, unlike the development of the sieve-plate, proceeds from the centre towards the periphery of the plate. In Fig. 18, Pl. XIX, a thin layer of callus is represented in process of being laid down against the wall of the tube; this layer soon increases in amount, generally starting from the sieve-plates, but sometimes arising separately in different parts of the tube[2]. Finally we obtain a stage such as that represented in Figs. 19, 23, Pl. XIX, in which the lumen is almost entirely obliterated.

iii. *Lateral sieve-plates.* On the lateral walls of the sieve-tubes, the places at which branching has occurred, or from which lateral connexions have originated, develop normal sieve-plates (Fig. 10, Pl. XIX). Sieve-plates are also developed on the cross walls of the rows of cells forming the cross connexions between the sieve-tubes. In many of these cases the

[1] Cf. Hill, l. c., 1901, II, Pl. XXXIII, Figs. 18, 23.

[2] Oliver, l. c., 1887.

perforations are so extremely small that it seems impossible that groups of threads can have preceded them, and perhaps the single threads originally present never divide in such sieve-plates to form groups. In some young lateral plates, however, groups of threads have been distinctly seen.

Besides these lateral sieve-plates, small isolated pads of callus are often found on the lateral walls of the sieve-tubes. A highly magnified example of one of these is shown in Fig. 22 *c*, Pl. XIX, and no threads or slime-strings can be seen in it. These lateral pads often appear to be sunk right inside the wall, as shown in Fig. 22 *a*, Pl. XIX, but sometimes each pad appears to surround a hole, the edges of this hole being continuous with the inner wall of the sieve-tube (Fig. 22 *d*, Pl. XIX). In the lateral walls of young sieve-tubes a few cases have been found in which a small callus-ring was present and a collection of deeply staining protoplasm was noticed lying adjacent to the ring. I think it probable that these pads were originally developed round pits, the remains of the pit being still visible in such cases as Fig. 22 *d*, Pl. XIX. As stated above[1], the pits in the longitudinal walls of the primary inner cortical cells were very rare and widely separated, and after the stretching of these cells to form sieve-tubes they naturally became still further apart, and, while some of them gave rise to cross connexions, others might perhaps be ruptured by the rapid growth of the surrounding walls, and callus-pads might then be formed round the old pit entrance. The first evidence of such a pad appears as a ring surrounding a pit; this ring then spreads over the pit-closing membrane, and finally a small mass of callus is developed which nearly or completely fills the pit.

iv. *Nature of the callus in Macrocystis.*

The callus in the primary pith filaments, secondary sieve-tubes, and hyphae of *Macrocystis* gives all the reactions characteristic of the ordinary callus found in the sieve-tubes of Phanerogams. In addition to those already enumerated by Oliver[2], London blue, like Water blue, gives it a fine blue colour, Congo red stains it a bright pink, and Thionin gives it a purplish tinge. The fact that Thionin also stains mucilage a similar, though deeper, purple is of interest in connexion with the conception of callus as a hydrated or mucilaginous form of cellulose. The frothy appearance of the callus in *Macrocystis*, an appearance which is increased in swollen material, is very striking, and an attempt to figure this is seen in a lateral pad shown in Fig. 22 *c*, Pl. XIX.

IV. Laminaria saccharina.

This species of *Laminaria*, though simpler than *Macrocystis* in many respects, is essentially similar as regards its general anatomy, while the histology of the two plants differs only in degree of development and complexity. I therefore propose to give but a short account of the anatomy of

[1] pp. 299-300.

[2] Oliver, l. c., 1887.

Laminaria saccharina, and then to pass on to its histology. The latter, more particularly the section dealing with the sieve-tubes, is of especial interest in connexion with the light which it throws on the evolution of the complex secondary sieve-tubes in *Macrocystis*, since these have hitherto been regarded as unparalleled among the Brown Algae.

(a) *General Anatomy.*

Stage i. Young plant. The intercalary growing region in a very young plant of *Laminaria saccharina* already reveals a differentiation into primary cortex and medulla, though secondary growth by means of a cortical meristem has hardly yet begun. A cross section of such a stage is shown in Fig. 46, Pl. XXI.

The cells of the outermost layer of the cortex are just beginning to divide tangentially and radially to form the secondary cortex, but the main part of the cortex is primary, and can be seen in longitudinal section to be composed of rows of cells showing a gradual transition, from almost isodiametric cells on the outside, to much more elongated ones near the medulla. In these last, which are rapidly being stretched longitudinally, the swelling of the walls is more or less obvious, causing the pits in the longitudinal walls to become much stretched, hence giving rise to cross connexions between the cells. Hyphae are found arising from the inner layers of the primary cortex in various ways. They may originate as small projections, either near the cross wall of a cell, or sometimes at other places on its longitudinal wall, but always on the side nearest the medulla. They grow inwards towards the medulla and, since even at this stage free ends are but rarely found, it seems certain that secondary connexions are speedily formed. They often grow straight across, and thus connect together two inner cortical cells on opposite sides of the medulla, and during their course they may or may not give off branches. Others again run but a short way into the medulla, and soon form secondary connexions with other hyphae.

The medulla, a more magnified transverse section of which is shown in Fig. 47, Pl. XXI, is composed of much elongated cells, dilated at the transverse septa and with longitudinal walls much swollen, owing to mucilaginous degeneration. The invading hyphae anastomose in the jelly thus formed, but they never form connexions with the elongated elements. There can be no doubt that these last are derived from the original central cells of the thallus, and I propose to call them 'primary pith filaments', as in *Macrocystis*. They probably function as the primary conducting-cells in the young plant and at this stage contain no callus.

Stage ii. Beginning of growth in thickness. The next stage investigated was found in material taken from the base of the lamina, in a young plant about eight inches in length.

A transverse section of this plant is seen in Fig. 48, Pl. XXI. A zone of secondary cortex of considerable size has been added by the meristematic division of the outermost cortical layer, and the cells produced by this meristem are seen to be arranged in radial rows. In longitudinal section the outer cells composing these radial rows are found to be isodiametric, while the inner ones are slightly elongated in a longitudinal direction. There is thus a gradual transition, in the length of the cells, between the secondary and primary cortex, the outer elements of the latter being now more elongated than in Stage i, while the innermost cells have by this time attained a considerable length.

In the cross walls of the secondary cortex the pits are arranged in a ring and are much deeper, and also sharper in outline, than are the more numerous and smaller pits in the primary cortex. The cells of the secondary cortex have also many more pits in their longitudinal walls than have those of the primary cortex.

Many interesting changes have occurred in the primary cortex since the last stage. The hyphae running into the medulla originate from several of the inner layers of the cortex and separate the cells of which these layers are composed, causing them in transverse section to appear as if arranged in radial rows. The outermost cells of these rows are square-ended and somewhat thick-walled, while the innermost cells have much more swollen walls and are dilated at the transverse septa.

The last of these elements pass gradually into the original medulla and, becoming very similar in appearance to the primary pith filaments, add considerably to its size. The increase in size of the medulla, due to the addition of these secondary elements, is readily seen on comparing Figs. 46 and 48, Pl. XXI. I propose to term these elements 'inner secondary sieve-tubes', but it must be borne in mind that they are produced by a late development from the already formed cells of the primary cortex, as are the so-called secondary sieve-tubes in *Macrocystis*, and in neither of these cases are they 'secondary' in the sense in which the term is used when speaking of secondary phloem in Phanerogams, not being formed, like that tissue, from the divisions of a secondary meristem.

The original medulla presents much the same appearance as that described in the last stage.

Stage iii. Adult stem. Fig. 49, Pl. XXI, represents a cross section of the adult stem magnified to the same degree as are Figs. 46 and 48.

It will be seen from the photograph that the secondary cortical tissue has greatly increased in amount, yet in longitudinal section the same gradual transition from periphery to centre is still noticeable. The extent of the primary cortex now bears a very small proportion to that of the secondary, and the hyphae arise from the outer as well as the inner layers, thus apparently increasing the number of elements in each radial

row. In longitudinal section the outermost cells of each radial row are seen to be much elongated and are square-ended, but there is a very gradual transition between these and the inner elements, which have much swollen longitudinal walls, are dilated at the septa, and, in their turn, pass gradually into the medulla. The question of the physiological function of the outer cells of the primary cortex is a very interesting one, and, as it is impossible to separate them from the inner elements, which are certainly sieve-tubes, I will say at once that I propose to call them 'outer secondary sieve-tubes'. The use of this term will be seen to be justified by an investigation of their histology.

Though a sharp line cannot be drawn between primary and secondary cortex, there is no reason to suppose that elements of secondary origin ever become transformed into outer secondary sieve-tubes; on the other hand I believe that these are entirely derived from the primary cortex, and that, when that tissue has been used up, no more sieve-tubes are formed [1]. The oldest specimen of *Laminaria saccharina* which I examined had a stem about three quarters of an inch in diameter, and, in a transverse section of this stem, there were found radial rows consisting of some ten or twelve elements surrounding the medulla. This number agrees with that observed in the radial rows of sieve-tubes in the plant figured, which had a diameter of less than half an inch.

The medulla is now a much more elongated oval in shape, and has considerably increased in size since the last stage, several of the inner secondary sieve-tubes from each radial row having become included in it.

A few elements, distinguished by their greater diameter and their position nearer the centre, probably represent the primary pith filaments. While these are always unbranched, the sieve-tubes formed from the primary cortex and added to the medulla often have lateral branches which in many cases arise at the level of the sieve-plate, recalling those cases in the young plant where some of the inner cortical cells gave rise to hyphae opposite their transverse septa. The three-armed sieve-plates thus produced are very striking (Fig. 36, Pl. XX).

The hyphae in the medulla have formed a much branched system, running in all directions in the jelly, and filling up the centre, but they have now no free ends.

Callus is largely developed in the primary pith filaments, and is also present in both the outer and inner secondary sieve-tubes, confirming the conclusion already arrived at that these elements are to be looked upon as true sieve-tubes. The extent of development of the callus was found to differ in winter and summer material, but reference to this point will be made later.

[1] Cf. *Macrocystis*, p. 300.

(b) *Histology.*

i. *Nature of cell-walls.*

The cell-walls in *Laminaria saccharina* are of an even more mucilaginous character than those of *Macrocystis*, and hence they stain very strongly with such reagents as Methylene blue, but more faintly with Congo red; with Iodine and Sulphuric acid the outer layers of the cell-wall rapidly turn blue, but the middle lamella first takes a yellow tinge, and afterwards turns blue like the rest of the wall.

The pit-closing membranes, as in *Macrocystis*, differ in their reactions from the general cell-wall. They become yellow when treated with Iodine and Sulphuric acid, and do not stain at all with Methylene blue or Congo red. The distribution of the pits in the cortex has been described above, but a more minute investigation of their nature shows that each primary pit is made up of a number of secondary pits; Figs. 1 and 2, Pl. XX, represent transverse walls of cells of the secondary cortex, and are drawn from preparations stained with Methylene blue. The general wall is deeply stained, but the pits appear as rounded areas in which are blue lines on an unstained ground. The blue lines separate the secondary pits from one another, while the spaces between them, which are left unstained, represent the pit-closing membranes of the secondary pits. The distribution of the connecting-threads is found to be practically confined to these unstained areas.

The sieve-plates, like the pit-closing membranes of the cortical cells, are of a more resistant nature than the general cell-walls. They turn yellow when treated with Iodine and Sulphuric acid, and do not stain with Congo red. After remaining for a short time in a solution of Methylene blue they present the appearance of a delicate reticulum, the meshes of which are blue, while the spaces between, which correspond to the areas traversed by the threads, remain unstained. The transverse walls of the young primary cortex, from which some of the sieve-plates are derived, give a similar reaction.

ii. *Protoplasmic continuity in cortical cells and hyphae.*

a. Cortex. In the outer secondary cortex the connecting-threads are always arranged in definite groups, almost, if not entirely, confined to the pits. A surface view of the cross wall of a cortical cell shows five to eight of these groups arranged in the form of a ring near the periphery of the wall, as seen in Fig. 3, Pl. XX. Each pit in the longitudinal wall is traversed by threads which here also are arranged in clusters (Figs. 6 and 7, Pl. XX).

In the inner secondary cortex the pits often extend much further towards the centre of the cross wall, and, when such cases are examined for threads, it is seen that, while groups of threads are present corresponding to the pits, a few isolated threads are also found in the other parts of the wall

(Fig. 4, Pl. XX). On the longitudinal walls the threads are restricted to well-defined pits.

In the primary cortex of the young plant no threads could be demonstrated, owing to this material having been preserved in spirit. No definite pits can be recognized in this region, and it is to be supposed that the threads would not be sharply differentiated into groups as in the secondary cortex, but that they would be evenly distributed as in the young sieve-plates.

b. Hyphae. At the point of origin of a hypha the threads are arranged in four or five rather indefinite groups, as seen in surface view in Fig. 12, and in section in Fig. 14, Pl. XX. In the winter material, callus was found to be developed to a small extent at the origin of the hyphae, and, as described below in the sieve-plates, each thread produces its own callus-rod (Figs. 13, 15, Pl. XX).

Connecting-threads were also seen in the transverse septa of many of the hyphae of the medulla, and here also callus was often found in the winter material (Figs. 22, 24, 25, 27, Pl. XX).

iii. *Development of the sieve-plates and obliteration of the sieve-tubes.*

(a) In the square-ended *outer secondary sieve-tubes*, derived from the outer layers of the primary inner cortex, the cross walls were found, in longitudinal section, to be traversed by numerous small groups of threads, seen in surface view to be arranged in various ways to form a larger pattern (Fig. 10, Pl. XX). In the summer material these threads stain with Safranin, in exactly the same manner as do the cortical threads, but in the winter material a deeper stain is taken by the threads of some of the inner of these sieve-plates, and, when a section is placed in London blue, each thread is found to be enclosed in its own callus-rod. Cases of callus formation in these secondary sieve-tubes are shown in section in Figs. 17, 18, 20, Pl. XX. The heads of the separate callus-rods are generally fused to form a small patch over each group of rods, but as a rule there is no great accumulation of callus. Fig. 21, Pl. XX, is taken from a very exceptional sieve-plate, situated on the innermost edge of a row of the outer secondary sieve-tubes, and consequently next the sieve-tubes of the medulla; a large mass of callus has been deposited on one side of the plate. In summer material a small amount of callus was twice found in elements in a position similar to this last.

On the lateral walls of the outer secondary sieve-tubes, pits traversed by threads are sometimes found (Fig. 18 *a*, Pl. XX). Larger lateral plates are present on the longitudinal walls of the inner of these elements, and some of these at any rate represent the origin of hyphae, while others are found at the origin of the larger cross connexions (Fig. 11, Pl. XX).

(b) *Sieve-tubes of the medulla.* There is a gradual transition from the

square-ended, more or less rudimentary outer sieve-tubes, to the elongated, swollen-walled sieve-tubes of the pith. In the younger sieve-plates belonging to the latter, the threads are rather difficult to demonstrate. In favourable material they were, however, well-stained, and surface views, showing their arrangement, are given in Figs. 28, 29, Pl. XX. Sometimes the threads are almost evenly distributed, sometimes they form small groups of ten or twenty, arranged in various ways, similar to the methods of distribution of the threads in the rudimentary sieve-tubes.

In a very few of the older sieve-plates these groups of threads have become converted into sieve-fields. Each group does not, as in *Macrocystis*, give rise to a single slime-string traversing a single hole in the sieve-plate, and enclosed in a callus-rod, but each thread of a group remains independent and forms a separate slime-string surrounded by its own callus-rod. The number and distribution of the slime-strings in such a stage as Fig. 32 *a*, Pl. XX, are found to be similar to those of the threads in Fig. 29, Pl. XX.

The first step towards the formation of the slime-strings is shown by the deeper staining power of the threads when treated by the Safranin method. At this stage, if the section be placed in London blue, a faint blue ring is seen, both in surface views and optical sections, to surround some of the threads (Fig. 30, Pl. XX). After this, perforation soon follows, and Figs. 31, 32, Pl. XX, represent surface views of sieve-plates riddled with holes. Each thread has become converted into a slime-string running through a hole in the plate, but in many cases the slime-strings have fallen out in the course of preparation of the section. Each hole is surrounded by callus which in surface view appears to spread over the sieve-field areas, but does not extend over the portions of the plate between the areas; Fig. 32 *b*, Pl. XX, is a small portion of Fig. 32 *a* more highly magnified, and showing the limits of the callus-patches. In optical section each slime-string can be seen in the thickness of the plate to be surrounded by a ring of callus, which is of course a tube in longitudinal section. The heads of the callus-rods soon fuse and form a small aggregation over the ends of each group (Figs. 36, 38, Pl. XX). Such aggregations are seen in surface view in three places in Fig. 31, Pl. XX.

At this stage the sieve-plate is at its fullest development. The sieve-tubes nearer the centre of the medulla, some of which represent the primary pith filaments, are in various stages of obliteration. Callus soon begins to accumulate over the original patches and from there spreads over the whole surface of the sieve-plate (Fig. 37, Pl. XX). A large mass of callus is found in the innermost elements and is generally much more developed on one side of the plate, probably, from analogy with *Macrocystis* [1], that side which is furthest from the apex (Fig. 39, Pl. XX). Not more than two sieve-tubes in this state are usually present in any radial section. In some of the

[1] p. 302.

oldest sieve-tubes, and also in a few of the hyphae, callus is formed throughout the length of the cell, and here, as in *Macrocystis*, it seems highly probable from its mode of formation that it is laid down directly by the protoplasm (Figs. 40, 41, Pl. XX).

All these stages, just described under the head of sieve-tubes of the medulla, were examined in winter material, but some obliterated sieve-tubes were found in summer material, and, after much swelling with various reagents, callus was also demonstrated in a few of the younger examples.

iv. *Nature of the callus.*

The callus of *Laminaria saccharina* does not appear to be all of the same kind. That found in the outer secondary sieve-tubes, in most of the hyphae, and in the young inner secondary sieve-tubes is only visible when stained with London blue, and it stains with that reagent in unswollen winter material, but gives no reaction with Water blue either in swollen or unswollen material.

It was stated above that in the summer little or no callus was to be seen. This statement applies to fresh material, but it was found that, after being left for a considerable time in some swelling agent, such as Potassium iodide and Iodine, a substance which stained with London blue was to be seen in several of the older sieve-tubes and plates, as well as in a few younger stages. In summer material rare examples of callus were found in the innermost sieve-tubes, which stained with Water blue or Russow's callus reagent, but in the winter material the callus present in all the older tubes, as well as that in such unusual cases as that figured in Fig. 21, Pl. XX, stains with these reagents.

It seems probable that callus in various states of hydration is present in *Laminaria saccharina*, for :—

(*a*) Most of that found in summer differs but little from the substance of the ordinary cell-wall, and only gives a characteristic callus reaction after further hydration with swelling reagents.

(*b*) In winter and in a few cases in summer the callus is sufficiently hydrated in the fresh material to stain with London blue. It becomes much more definite after swelling for some time, but it was never found to stain with Water blue.

(*c*) Finally, the most hydrated form of callus is similar to that found in *Macrocystis*, and stains with Water blue as well as with London blue. It was only found in the sieve-tubes of the medulla and in the exceptional case mentioned above [1], and shown in Fig. 21, Pl. XX. This fully hydrated callus is hardly ever formed in summer.

[1] p. 311.

V. Sieve-plate Development and Callus Formation in *Macrocystis* and *Laminaria*.

In this section I wish first to point out how completely the observed series of changes in the development of the sieve-plate in these two plants harmonizes with the interpretation of sieve-plate development in *Pinus* given by Hill.[1] From these researches it appears probable that the boring-out of the protoplasmic threads to form slime-strings is due to ferment action. In *Macrocystis* and *Laminaria*, after the manner described for *Pinus*, the passage of the ferment down the young threads may be traced by the alteration of the staining properties of the protoplasm and by the change of the cell-wall into callus. It appears in *Macrocystis*[2], as in *Pinus*[3], that the ferment produced in any given sieve-tube is only able to act as far as the middle lamella of the wall which separates this sieve-tube from an adjoining one, a fact which lends support to the view that the young threads are interrupted at the middle lamella.[4] Structures which may be compared to the median node of the threads of *Pinus* and to the median nodules of the slime-strings in that plant[5] were occasionally met with in *Macrocystis*[6], and might have been more distinctly seen in better preserved material.

The various states of callus found occurring in *Laminaria saccharina*[7] confirm the idea that that substance is a hydrated form of cellulose, and it is suggested that it is in a different degree of hydration in each of the states described. In summer there is present in the sieve-tubes a substance which will not react to any known callus-stain, but by hydration with swelling reagents it is possible to induce it to stain with London blue. Further swelling of callus which stains naturally with London blue causes it to stain more deeply with that reagent, but can never induce it artificially to attain a sufficient degree of hydration to stain with Water blue, though sometimes it can be made to react when treated with Russow's callus reagent after swelling for a considerable period. In winter all degrees of hydration of the callus were found in the various elements of *Laminaria*.

It appears that in the Laminariaceae, as in Phanerogams,[8] callus can originate either by a change in the already formed cell-wall or by deposition from the protoplasm; in the formation of callus-rods in the sieve-plate it seems highly probable that the callus is produced by a change in the cell-wall.

But with regard to the accumulation of callus in masses at the sieve-

[1] Hill, l. c., 1901, II, pp. 594-596.
[2] Cf. Figs. 14, 15, Pl. XIX.
[3] Hill, l. c., 1901, II, Figs. 18, 23, Pl. XXXII.
[4] Cf. Gardiner, Proc. Camb. Phil. Soc., xiv, 1907, p. 209.
[5] Hill, 1901, Fig. 12, Pl. XXXII, and Fig. 20, Pl. XXXIII.
[6] Cf. p. 304.
[7] Cf. p. 313.
[8] Hill, l. c., 1901; and Hill, Notes on the Histology of the Sieve-tubes in certain Angiosperms, Ann. of Bot., 1903, p. 267.

plates, and its formation throughout the length of all classes of sieve-tubes I have been unable to confirm Oliver's conclusion that here also callus is due to mucilaginous degeneration of the cell-wall. Oliver may have been led to this view by working on herbarium material, but in the course of the present research all the evidence obtained from *Macrocystis* and *Laminaria* has pointed to the conclusion that the callus is directly laid down by the protoplasm, and thus supports the views of Gardiner [1] and Hill [2]. It is only necessary here to refer to the description on p. 302, and to Figs. 23, 27, and 17–20, Pl. XIX, Figs. 21 and 40–42, Pl. XX, in order to show how difficult it would be to harmonize such appearances with the view that the callus is produced from the already formed cell-wall. No cases were observed in which the callus was seen to fade gradually into the cell-wall, as described by Oliver [3], and as also shown by Hill [4] in an abnormal case in a sieve-tube in *Pinus*.

As in *Pinus*, the unaltered portions of the old cross wall are still visible in the oldest sieve-plate and are seen in longitudinal sections as beads, of a glistening appearance, which do not stain with callus-stains. The callus-cushion attains a very large size (Fig. 19, Pl. XIX), but the dimensions of the original cell-wall diminish very slightly, if at all, and it is impossible to believe that the minute layer of cellulose which could be thus accounted for can be responsible for the production of such a large mass of callus.[5]

VI. Comparison of the Anatomy and Histology of *Macrocystis pyrifera* and *Laminaria saccharina*.

In the young plants of both *Laminaria* and *Macrocystis* the original central cells become stretched and give rise to elongated elements which have been described as 'trumpet hyphae' by other authors.[6] As suggested above, it seems better to term them 'primary pith filaments'. They probably function for conduction in the young plants; in *Macrocystis* they are very early obliterated by callus, but in *Laminaria* this does not take place till a very much later stage.

The stretching process begun in the central cells of the thallus is later continued in the primary cortex. In *Laminaria* the cells of this tissue elongate and are transformed, without further cross division, into secondary sieve-tubes; in *Macrocystis* each cell divides transversely several times during the process of elongation, and here also all the primary cortex finally gives rise to secondary sieve-tubes. Thus in both plants the secondary

[1] Gardiner, Observations on constitution of callus, Proc. Camb. Phil. Soc., v, 1885, p. 230, and cf. Gardiner and Ito, Mucilage-secreting hairs in *Blechnum* and *Osmunda*, Annals of Botany, i, pp. 33 et seq., and p. 39 *a*, Figs. 34, 36, 41, 43.

[2] Hill, l. c., 1901, pp. 597–600, and cf. Figs. 22, 26, 27, Pl. XXXIII.

[3] Oliver, l. c., 1887, Figs. 2, 10, 11, Pl. I and II.

[4] Hill, l. c., 1901, p. 598, Fig. 25, Pl. XXXIII.

[5] Ibid., p. 599.

[6] Pp. 298 and 307.

sieve-tubes together with the primary pith filaments represent the whole of the young thallus, the entire cortex of the adult being of later origin.

Growth in thickness in *Macrocystis*, as in *Laminaria*, is due to the production of this late cortex by the divisions of an outer cortical meristem, and no other meristem is ever present. When the primary cortex has been entirely transformed to sieve-tubes, there is no reason to suppose that further sieve-tubes are produced from the secondary cortical cells in either plant. Hence the increase in size of the medulla, after additional sieve-tubes have ceased to be formed, must be ascribed to the swelling of the walls and the greater diameter of the elements already present, also to the numerous hyphae which grow in from the inner cortical layers and form an anastomosing system throughout the medulla.

In *Laminaria saccharina* the innermost secondary sieve-tubes, produced from the primary cortex, have much swollen walls, and resemble in every particular the primary pith filaments. On the other hand, even in the oldest stem examined, the outermost secondary sieve-tubes remain more or less rudimentary, but there can be no doubt that both kinds of elements are to be regarded as homologous with the well-known sieve-tubes of *Macrocystis*. In the latter, however, the secondary sieve-tubes have attained a far greater degree of development; they differ in size and general appearance from the primary pith filaments, and there is no gradual transition as in *Laminaria*. This difference in *Laminaria* may well be explained as due to the more gradual growth in the latter plant, there being no pause in the differentiation of the elements of the original thallus, but merely a slow diminution in amount of elongation, from the central cells to those of the periphery. It is probable that the greater development of the sieve-tubes in *Macrocystis* is due to its habit of growth and to the considerable length attained by the stem, as has already been suggested by Oliver [1] and Wille [2].

In longitudinal section it is of interest to compare the appearance of an adult stem of *Laminaria* with that of a young plant of *Macrocystis* in the stage described as *Stage* ii [3]. At that age it is found that there are only two or three layers of sieve-tubes as yet developed from the primary cortex, while the still rapidly elongating square-ended cells of the remainder of the cortex are not yet transformed into sieve-tubes. While the differentiated sieve-tubes are here to be compared with those of the adult *Laminaria* which become part of the medulla, the primary cortical cells are in appearance not unlike the outermost sieve-tubes in *Laminaria*, which always remain rudimentary.

The main points in connexion with the development of the sieve-plate in the two plants are strikingly similar, but some difference in detail is found in *Macrocystis* which may be largely due to the far greater size

[1] Oliver, l. c., 1887. [2] Wille, l. c., 1897. [3] p. 299.

of the sieve-plate in that plant. In *Laminaria* the single threads traversing the young plate are arranged in more or less well-defined areas. Each thread develops its own callus-rod and gives rise to a separate slime-string, and, though the heads of a group of these rods are often united, the rods themselves always remain distinct throughout the thickness of the plate. In other words, each thread produces a separate perforation in the sieve-plate. In *Macrocystis* the threads are homogeneously distributed in the very young sieve-plate, and are not confined to any special areas. Each original thread soon divides to form a group, and in the first stage of callus formation each thread of such a group has its own callus-rod; but from each group of threads with their separate callus-rods is finally developed a single slime-string enclosed in one callus-rod. In other words, each thread of a young sieve-plate eventually gives rise to a separate perforation in the sieve-plate, but, in between the original and the final state, a stage is found in which a group of threads is present, derived by the division of the single thread, and giving rise by fusion to a single slime-string.

In *Macrocystis* and *Laminaria* the old sieve-tubes are obliterated in very much the same way by the deposition of callus. In the former plant the callus begins to accumulate at the centre of the sieve-plate in a single mass and spreads thence to the periphery; in the latter, callus begins to be formed over each area of threads in numerous places on the sieve-plate, and then spreads in every direction over its surface.

Rarely in the summer, more often in the winter, callus is found in *Laminaria* which has the same properties as that of *Macrocystis*, but callus in a lower state of hydration is more often present in *Laminaria*.

The structure of the general cell-walls and of the pit-closing membranes is essentially the same in the two plants.

VII. Other Species examined.

I have examined various other species of the Laminariaceae, by way of supplementing the results obtained during the above research, and I propose to refer to them shortly here.

Sacchoriza (*Laminaria*) *bulbosa*, De la Pyl., has no distinct medulla and no sieve-tube-like elements. Some elongated cells, with living contents and very thick walls, are found in the central part of the stem; these do not appear to anastomose with each other or with other elements.

Laminaria digitata, Lamour., of which the material examined was preserved in summer, is very similar in structure to *Laminaria saccharina*. Its stem is differentiated into cortex and medulla, and numerous sieve-tubes are found in the medulla. The inner cortical layers, which resemble in position and appearance the outer secondary sieve-tubes of *L. saccharina*, give rise to hyphae which grow into the medulla. In the innermost cells of this tissue exceptional cases of callus formation were found; in two

of these the callus stained with Water blue or London blue, and in two only with London blue. In the sieve-tubes of the pith and in the cross walls of several of the hyphae numerous callus-rods were found, some of which stained only with London blue, while a few of the more central elements contained callus which gave the characteristic reaction with Water blue. Threads traversing the young sieve-plates, and slime-strings in the older sieve-plates, were satisfactorily demonstrated in a few cases, and several inner sieve-tubes, probably primary pith filaments, were found to be entirely filled with callus which stained with London blue and Russow's callus reagent, and sometimes with Water blue.

It is probable that, if well-preserved material were examined, *Laminaria digitata* would yield results similar to those obtained from *L. saccharina.*

Alaria esculentea, Grev. Some material pickled in Formalin was examined by the Safranin method. The results obtained were very similar to those of Wille[1]: callus was seen in the sieve-tubes of the medulla, but no secondary sieve-tubes were demonstrated.

Nereocystis Luetkeana, P. and R. Some spirit material was investigated, and the conditions obtaining in the sieve-tubes appeared to be very similar to those present in *Macrocystis.* In the primary pith filaments callus was found in large quantities, often entirely obliterating the lumen. In the young secondary sieve-plates callus was seen in surface view to appear in patches as in *Macrocystis*; in older stages it was seen to accumulate in masses, at first as in *L. saccharina.* Soon, however, it became heaped up in the centre by deposition from the dense central mass of protoplasm, and then spread over the whole sieve-plate, forming a large cushion. In a few old sieve-tubes callus was seen to be formed throughout the length of the tube, and stages like that seen in Fig. 23, Pl. XIX, were observed, in which there could be no doubt that the callus was deposited by the protoplasm.

VIII. Summary and Conclusions.

I. There can be no doubt that the 'trumpet hyphae' in *Macrocystis pyrifera* and *Laminaria saccharina* are to be looked upon as true sieve-tubes. They represent the modified, original central cells of the thallus, and may be termed 'primary pith filaments'. Though they differ as to their degree of development, they are certainly homologous with the secondary sieve-tubes of *Macrocystis*, which are similarly derived from the modified primary cortex of the young thallus.

II. Secondary sieve-tubes have also been demonstrated in *Laminaria saccharina* and are probably present in *Laminaria digitata.* These, like the secondary sieve-tubes in *Macrocystis*, represent the whole of the original

[1] Wille, l. c., 1897.

primary cortex and, both in position and mode of development, are undoubtedly homologous with those well-known elements. The greater development of the conducting organs in *Macrocystis* and *Nereocystis* is probably due to the habit of those plants.

III. The histology of the sieve-plates, in the primary pith filaments and secondary sieve-tubes, is essentially the same. Threads are found traversing the young sieve-plate, and each gives rise in the older plates, apparently by means of ferment action, to a slime-string enclosed in a rod of callus. In *Macrocystis* each original thread first divides to form a group, and each thread of a group forms its own callus-rod, but finally, by fusion of these, only one slime-string is produced from each group. The older sieve-plates are obliterated by the deposition of callus in large masses over their surface, and callus is also formed throughout the length of the old sieve-tubes.

IV. Callus is to be looked upon as a hydrated form of cellulose, and is found in *Laminaria saccharina* and *L. digitata* in various states of hydration. It appears to be produced in the young sieve-plates by the action of a ferment on the already formed cell-wall, but is afterwards accumulated by deposition from the protoplasm, both on the surface of the sieve-plate and on the lateral walls of the tube.

V. It is interesting to note how fully the histology of the sieve-tubes agrees with that of the sieve-tubes of Phanerogams. It is observed that, at the advent of the callus, a simultaneous increase of staining capacity becomes noticeable in the threads, and, as in *Pinus* [1], it is suggested that the development of the sieve-plate is a function of ferment action. There is one point of contrast between the method of obliteration of the sieve-tubes in the Laminariaceae and *Pinus*. In *Pinus* [2] the heads of the slime-strings were found to be still visible on the free edge of the callus-cushions, and the path of the slime-strings could be traced throughout the callus-mass; but in *Macrocystis* and *Laminaria*, on the other hand, the callus is laid down by the protoplasm of the sieve-tube, over the heads of the slime-strings, so that they are buried by the overlying callus, and no perforations can be traced through the pad [3].

VI. It has been shown that in young stages of *L. saccharina* the cells of the hyphae become secondarily attached to those of the primary cortex, and that this phenomenon also probably occurs in *Macrocystis*.

VII. Protoplasmic connecting-threads have been demonstrated throughout the tissues of *Macrocystis pyrifera* and *Laminaria saccharina*, but it is impossible to be certain of their formation in the case of secondary attachments. Their demonstration in cells not genetically connected would

[1] Hill, l. c., 1901.

[2] Fig. 19, Pl. XIX, Fig. 39, Pl. XX.

[3] Ibid., 1901, II, Fig. 14, Pl. XXXII.

be interesting in connexion with recent theories of the development of connecting-threads [1].

The remarkably close resemblance found at every stage between the development of the sieve-tubes in the Laminariaceae and Phanerogams [2] would almost have sufficed some years ago to convince us of the truth of the homologous theory of alternation of generations, but, though it may still be interesting in connexion with that theory, the present knowledge of cases of homoplasy is so rapidly accumulating that such a resemblance can no longer be regarded as having much weight.

EXPLANATION OF FIGURES IN PLATES XIX–XXI.

Illustrating Miss Sykes's Paper on *Macrocystis pyrifera* and *Laminaria saccharina*.

PLATE XIX. *Macrocystis pyrifera*.

The lenses used were Swift's, Zeiss', and Watson's $\frac{1}{12}$ apoc. with six and eight compensating oculars. Unless otherwise specified, the preparations were stained by the Safranin method followed by London blue. The blue colour in the figures represents callus.

Fig. 1. Cross wall of a cortical cell in surface view, showing arrangement of pits; note curious striations in the wall itself. (Stained watery Methylene blue.) × 600.

Fig. 2. Cross wall of a cortical cell in surface view, showing the ends of protoplasmic threads as dots on the pit-closing membranes. × 600.

Fig. 3. Longitudinal section of a cell of the inner cortex. Pits are seen in section in the end-wall, and connecting-threads traverse the pit-closing membranes both there and in the solitary pit (*x*) found in the lateral wall. × 600.

Fig. 4. Longitudinal section of part of the lateral wall of an inner cortical cell. An unusual case showing several lateral pits quite near together. × 750.

Fig. 5. Surface view of a very young sieve-plate, from the outermost row of secondary sieve-tubes in an old stem. The ends of the threads are seen as fine dots and are arranged singly except on the periphery of the plate, where a few groups are seen. × 600.

Fig. 6. Surface view of a slightly older sieve-plate, being the second element in a radial row, counting from the outer edge. The threads are arranged throughout in groups of four, five, and six. (Stained Safranin and Acid violet.) × 600.

Fig. 7. Surface view of a sieve-plate, showing progressive differentiation from the centre to the periphery. A mass of deeply staining protoplasm hides the centre of the plate, where probably only single threads would have been found. A few single threads are still visible round this mass, but most of them have each given rise to groups of two and three threads. Nearer the outside, groups of five are present, and those represented as surrounded by a ring have already developed callus, while on the extreme periphery single slime-strings, each formed from one of these groups and enclosed each in a single callus-rod, are found. × 600.

Fig. 8. Surface view of part of a sieve-plate; a slightly older stage than Fig. 7, with callus represented blue. In the centre are seen groups of threads (*a*) which have not yet developed callus Around these are groups of more deeply staining threads each enclosed in a callus-spot (*b*); nearer the outside the callus-spots forming a group have united by their edges to produce a ring (*c*); *d* is a case in which the threads, callus, and portion of wall enclosed by such a ring have begun to break down, and have given rise to two slime-strings; on the periphery the process of boring out is complete and a single slime-string (*e*) has been produced from each original group and is surrounded by a thick ring of callus. In one place (*f*) the callus has begun to spread over the portions of the wall which lie between the perforations. × 600.

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[1] Gardiner, Roy. Soc. Proc., 1900. See also Meyer, A., Die Plasmaverbindungen und die Fusionen der Pilze der Florideenreihe, Bot. Zeit., 1902, p. 139.

[2] Hill, l. c., 1901 and 1903.

Fig. 9 is a diagrammatic representation of the changes undergone by a single group of threads. (Letters as in Fig. 8.)

Fig. 10 is a lateral sieve-plate seen partly in section, partly in surface view. Single callus-rods are seen traversing the sieve-plate. × 750.

Fig. 11. Very young sieve-tube in longitudinal section showing single threads traversing the sieve-plate. × 600.

Fig. 12. As Fig. 11, but the central portion of each thread more deeply stained. × 600.

Fig. 13. Young sieve-plate, showing single threads in some places, and groups of twos and threes in others, in longitudinal section. × 750.

Fig. 14. First step in callus formation, in a young sieve-plate in which the threads are arranged throughout in groups. Callus is found in three places, but in each case has only been formed on one side of the middle lamella. × 750. At (*a*) two pairs of deeply staining threads are seen, in one of which the change has not yet reached across the middle lamella.

Fig. 15. An unusual case of a sieve-plate in which development has proceeded further on the centre than on the periphery. On the left of the section are seen groups of two threads, and in one of these groups a median node is visible on each thread. Next to this group comes one made up of three threads each of which has its own callus-rod; next to this, one in which two slime-strings are present, the formation of which is not yet complete across the middle lamella; in the centre is a case in which the single slime-string is nearly complete, but across the middle lamella two separate darkly staining threads still stretch. On the right of the plate the outermost pit but one shows a case in which callus and slime-string formation is complete on the upper side, while threads without callus are still present on the under side. × 1,000.

Fig. 16. In this sieve-plate fully formed slime-strings, each enclosed in a callus-tube, are seen on the periphery in longitudinal section. In between the periphery and the centre all stages in their formation are present. At (*a*) is seen a very common phenomenon: the half-formed slime-strings on one side dragged out of the pits during the processes of staining, &c. In many places in this sieve-plate the callus has spread over the areas between the perforations. The protoplasm of the sieve-tube is massed in the centre. × 750.

Fig. 17. First stage in the obliteration of a sieve-tube: an accumulation of callus has occurred at the centre of the plate. × 750.

Fig. 18. An old sieve-tube in which is seen the commencement of callus deposition on the side-walls. This preparation was stained to show the much swollen inner layer of the wall. *ml* = middle lamella; *x* = lateral plate, finely perforated. (Stained Thionin and London blue.) × about 500.

Fig. 19 represents one of the oldest sieve-tubes in longitudinal section. Obliteration of the sieve-plate is here complete and callus has also been laid down in considerable quantity on the lateral walls. At (*a*) a portion of protoplasm is seen entirely enclosed by callus, clearly showing that the callus cannot here be due to an alteration of the wall. × about 450.

Fig. 20. *a*, *b*, *c*, *d*, are cross sections of sieve-tubes showing various stages in the blocking of their lumina with callus. × 600.

Fig. 21. As Fig. 20, showing lumen almost obliterated. × 600.

Fig. 22. *a*, *b*, *c*, *d*, are cross sections of sieve-tubes showing the formation of lateral pads of callus. The pad in 22 *d* is seen to surround a hole, and the edges of the hole are continuous with the inner edge of the sieve-tube wall. Fig. 22 *c* is one of these lateral pads very much enlarged, and an attempt is made to show the flocculent nature of the callus.

Fig. 23 is a longitudinal section of one of the innermost sieve-tubes and shows the unequal deposition of the callus on the lateral walls.

Fig. 24. A longitudinal section of the cross wall of one of the anastomosing hyphae of the medulla. The connecting-threads are shown as fine lines traversing the wall. × 600.

Fig. 25. As Fig. 24, showing callus formation at a cross wall.

Figs. 26–32 are drawn from the young apex of *Macrocystis* preserved in spirit. Primary pith filaments.

Fig. 26. A longitudinal section of a young primary pith filament in which no callus has yet been formed. The knobs on either side of the sieve-plate probably represent developing slime-strings. × 600.

Fig. 27. A slightly older element in which the development of callus has begun both at the sieve-plate and on the lateral walls of the sieve-tube. × 600.

Fig. 28. A longitudinal section of a sieve-plate on which callus has begun to accumulate. (Thionin and London blue.) × 600.

Fig. 29. A longitudinal section of a primary pith filament in which an aggregation of callus has been formed at the centre of the sieve-plate; cf. Fig. 17. × 600.

Fig. 30. An older element in which a large mass of callus has been deposited on the lower side of the sieve-plate, that is the side furthest from the apex. × 600.

Fig. 31 is taken from a case in which a considerable amount of callus has accumulated on both sides of the sieve-plate and has also been deposited down the sides of the tube; cf. Fig. 23. (Stained Thionin and London blue.) × 600.

Fig. 32. A cross connexion between two primary pith filaments; callus has been formed at the cross walls and is greater in amount at the wall which is nearer to the centre of the stem. × 750.

PLATE XX. *Laminaria saccharina* (adult preserved i winter).

Unless otherwise specified, the preparations from which the following figures are taken were stained by the Safranin method, followed by Aniline blue and London blue.

Fig. 1. Surface view of the cross wall of a cell of the middle cortex. Five large primary pits, each divided into a number of secondary pits, are present. The ordinary wall is stained deep blue, the pit-closing membranes left white. (Stained Methylene blue.) × 600.

Fig. 2. As Fig. 1, from the inner edge of the middle cortex. × 600.

Fig. 3. Surface view of the cross wall of an outer cortical cell, showing the ends of the connecting-threads arranged in gronps, each group corresponding to one of the primary pits in Figs. 1 and 2. × 600.

Fig. 4. Surface view of the cross wall of an inner cortical cell just outside the secondary sieve-tube layer. The threads here are arranged in groups mostly confined to the pits, but a few are also seen in the central part of the wall. × 600, and drawing slightly enlarged.

Fig. 5. Longitudinal section of a cell of the outer cortex, showing four pits in an end-wall, and connecting-threads as fine lines traversing the pit-closing membranes. × 600.

Fig. 6. Two pits in a longitudinal wall of a middle cortical cell, showing the threads in longitudinal section. × 750.

Fig. 7. A single pit on the lateral wall of an inner cortical cell, in longitudinal section. × 750, and drawing much enlarged.

Fig. 8. Longitudinal section of a cell of the middle cortex passing through two pits in an end-wall. × 600.

Fig. 9. Longitudinal section of a cell of the inner cortex, situated just outside the secondary sieve-tube layer. The end-wall is traversed by threads which are arranged in rather indefinite groups. Note very shallow pits. × 750, and drawing much enlarged.

Fig. 10. Surface view of the cross wall of a young outer secondary sieve-tube, showing the ends of the threads arranged in a star-like pattern. × 600.

Fig. 11. Surface view of the point of origin of a hypha from an outer secondary sieve-tube. × 600.

Fig. 12. As Fig. 11.

Fig. 13. Surface view of the point of origin of a hypha from an outer secondary sieve-tube, showing callus. × 600.

Fig. 14. Surface view of the point of origin of a hypha after callus formation.

Fig. 15. Longitudinal section of an outer secondary sieve-tube, showing the cross wall, partly in section and partly turned up so as to appear in surface view. The threads are arranged in small groups. × 600.

Fig. 16 as Fig. 15. × 600.

Fig. 17. Longitudinal section of an outer secondary sieve-tube situated the third from the periphery in a radial row. Callus has been formed, and each thread has its own callus-rod, which can be seen traversing the thickness of the plate, but the heads of the threads are fused together to form a mass. (*a*) = origin of hypha. × 600.

Figs. 18, 19, 20 are similar cases to Fig. 17. (*a*) = origin of hyphae at which callus formation has occurred. × 600.

Fig. 21 is an unusual case in which a large accumulation of callus has taken place on one side of a sieve-plate in an outer secondary sieve-tube. The element from which this figure was taken was next to the medulla. × 600.

Fig. 22. Surface view of a cross wall of an anastomosing hypha from the medulla. × 600.

Fig. 23, as Fig. 22 displaced in longitudinal section.

Figs. 24, 25, 26, 27. Longitudinal sections of hyphae in the medulla, in the cross walls of most of which callus formation has occurred. × 600.

Fig. 28. Surface view of a young inner secondary sieve-plate from the periphery of the medulla. Hyphae are seen cut during their course in the walls, and a small sieve-plate is also seen in surface view in the left-hand corner, and probably represents the cross wall of a large hypha. The threads seen in end view in the large sieve-plate are confined to certain areas in the wall and are arranged in a star-like pattern. × 750.

Fig. 29. Surface view of a sieve-plate from an inner secondary sieve-tube. The ends of the threads are arranged in smaller groups than are those in Fig. 28. × 750.

Fig. 30. Surface view of a slightly older sieve-plate from the medulla. A few of the threads stain more darkly than the rest, and are each enclosed in a ring of callus. × 600.

Fig. 31. Surface view of a portion of a sieve-plate in which perforation is complete. Each thread has become transformed to a slime-string, and a faintly staining ring of callus was visible round each string, but is only indicated in the figure by a black line. The callus has already begun to accumulate in three places. × 600.

Fig. 32 *a*. A slightly later stage than Fig. 31 in surface view. The callus has now spread over all the areas of the plate which were originally traversed by threads, but has not yet been formed in the other portions of the plate. × 600.

Fig. 32 *b* is a very much enlarged representation of a small portion of the plate, in which the faintly staining callus is coloured blue, and a small area left unattacked is left white. (In Figs. 31 and 32 the slime-strings are seen to have fallen out of most of the holes during the process of preparing the sections.)

Fig. 33. Longitudinal section of a sieve-tube of the medulla, showing the sieve-plate traversed by groups of threads. × 600.

Figs. 34 and 35 as Fig. 33.

Fig. 36. Longitudinal section of 'three-armed sieve-plate' from the medulla. Callus has been formed in both terminal and lateral plates, each thread being enclosed in its own callus-rod, and the heads of each group of threads being fused together. × 600.

Figs. 37 and 38. Longitudinal sections of two sieve-plates from the medulla, showing callus formation in each group of threads and accumulation above the groups to form small cushions. Fig. 38 *b* shows one of these cushions much enlarged. × 600.

Fig. 39. An old sieve-tube in longitudinal section (× 600), showing a large accumulation of callus on one side of a sieve-plate. × 600.

Fig. 40. A similar case in which callus formation has also commenced down the sides of the sieve-tube. × 600. This figure should be compared with Figs. 23 and 31, Pl. I, and Fig. 41, Pl. II.

Fig. 41. Longitudinal section of a similar sieve-tube, showing callus formation on the lateral walls obliterating the lumen in some places. × 600.

Fig. 42, *a*, *b*, *c*, *d*. Transverse sections of sieve-tubes and primary pith filaments from the medulla, showing the lumina in various stages of being blocked by callus. × 600.

Fig. 43. Transverse section of a sieve-tube, showing origin of lateral branch or of a pulled-out cross connexion. Callus has been formed as on the end-walls of the sieve-tubes. × 750.

PLATE XXI.

(From photographs, which are described in text.)

Fig. 44. Cross section of young stem of *Macrocystis pyrifera*.

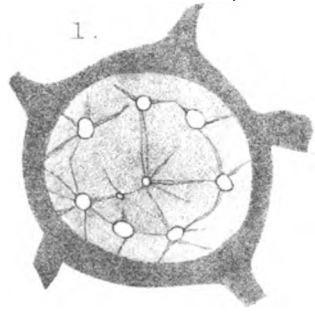
Fig. 45. Part of cross section of old stem of *Macrocystis pyrifera*. The radial rows of secondary sieve-tubes are seen on the periphery of the medulla.

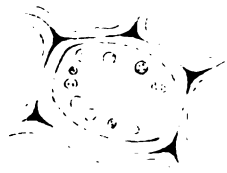
Fig. 46. Cross section of very young plant of *Laminaria saccharina*.

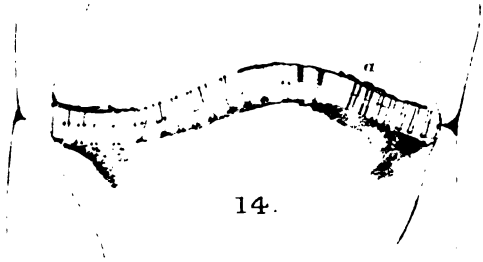
Fig. 47. Central portion of Fig. 3, much more highly magnified.

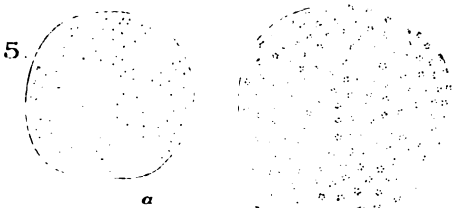
Fig. 48. Cross section of young plant of *Laminaria saccharina*.

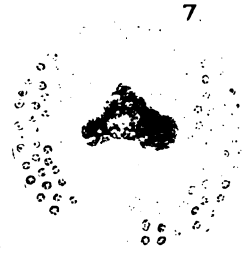
Fig. 49. Cross section of old plant of *Laminaria saccharina*, showing radial rows of sieve-tubes on the periphery of the medulla, and a few large sieve-tubes nearer the centre of the medulla. Figs. 3, 5, and 6 are all magnified to the same extent, and illustrate the tremendous increase in amount of the secondary cortex.

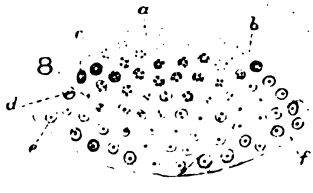


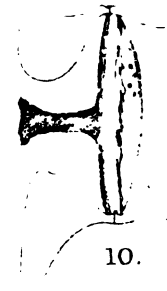


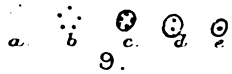


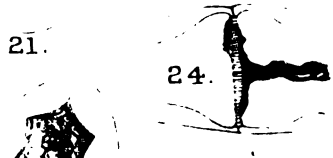


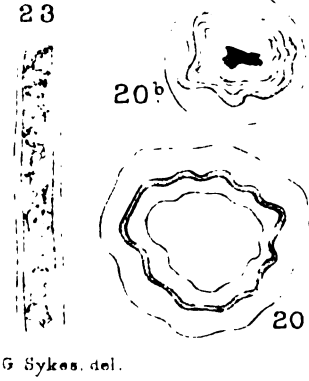


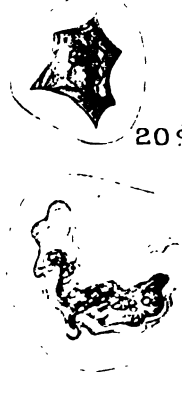


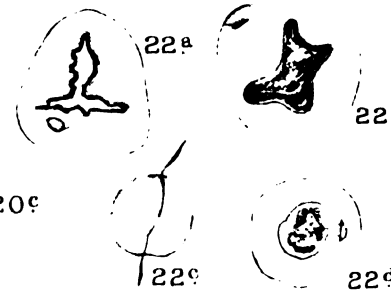












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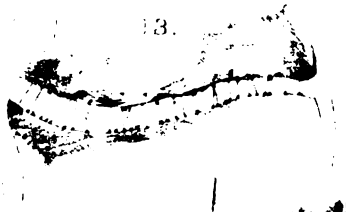

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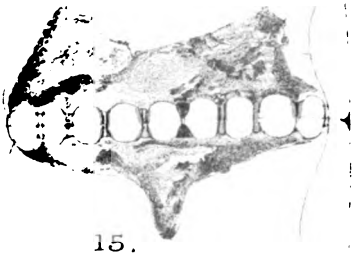

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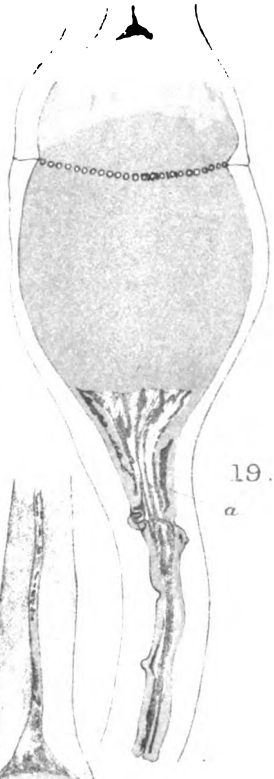

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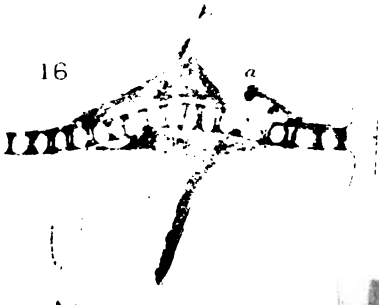

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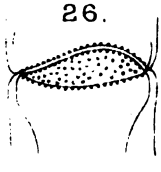

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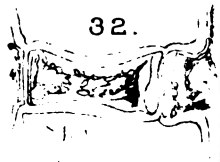

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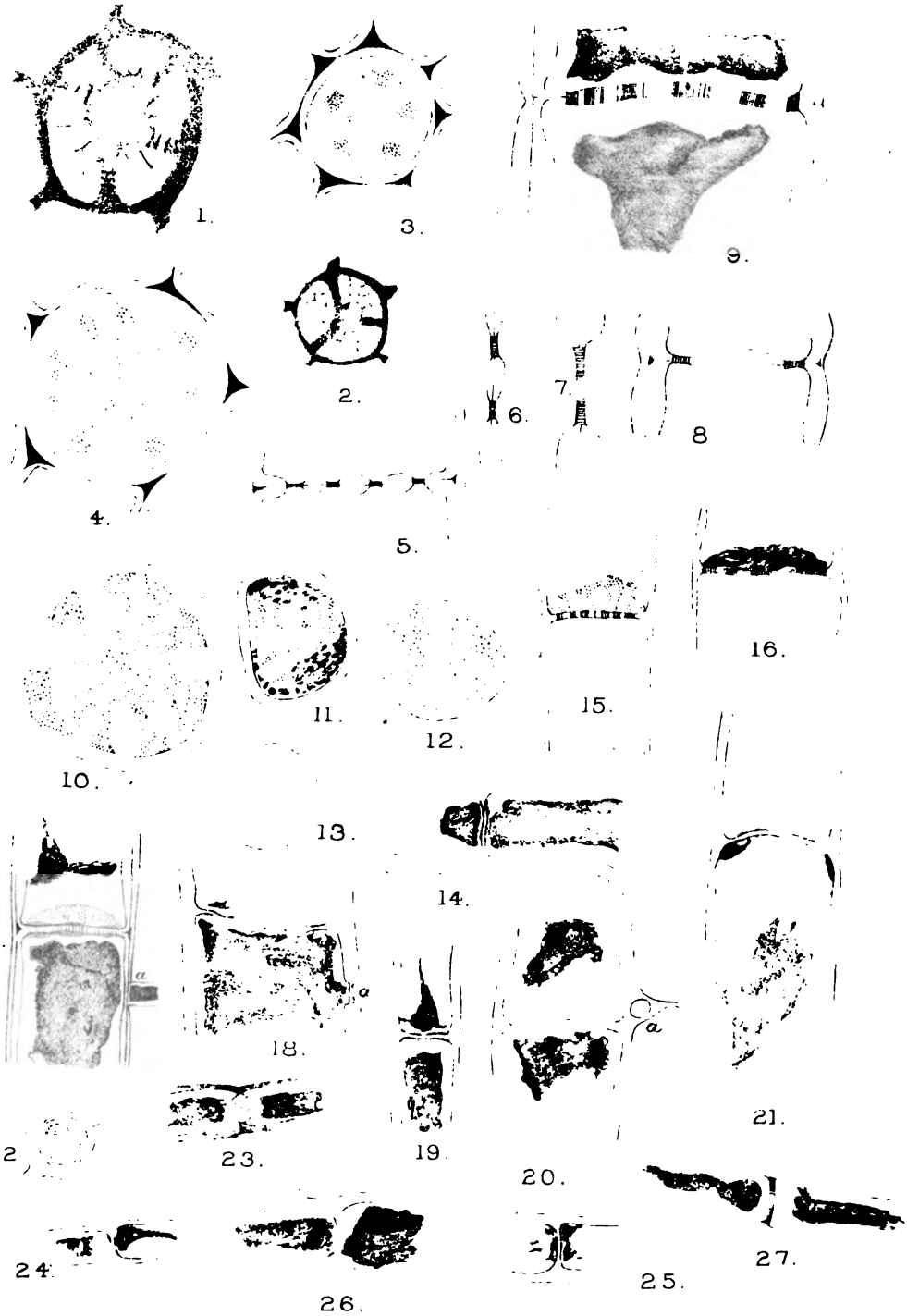
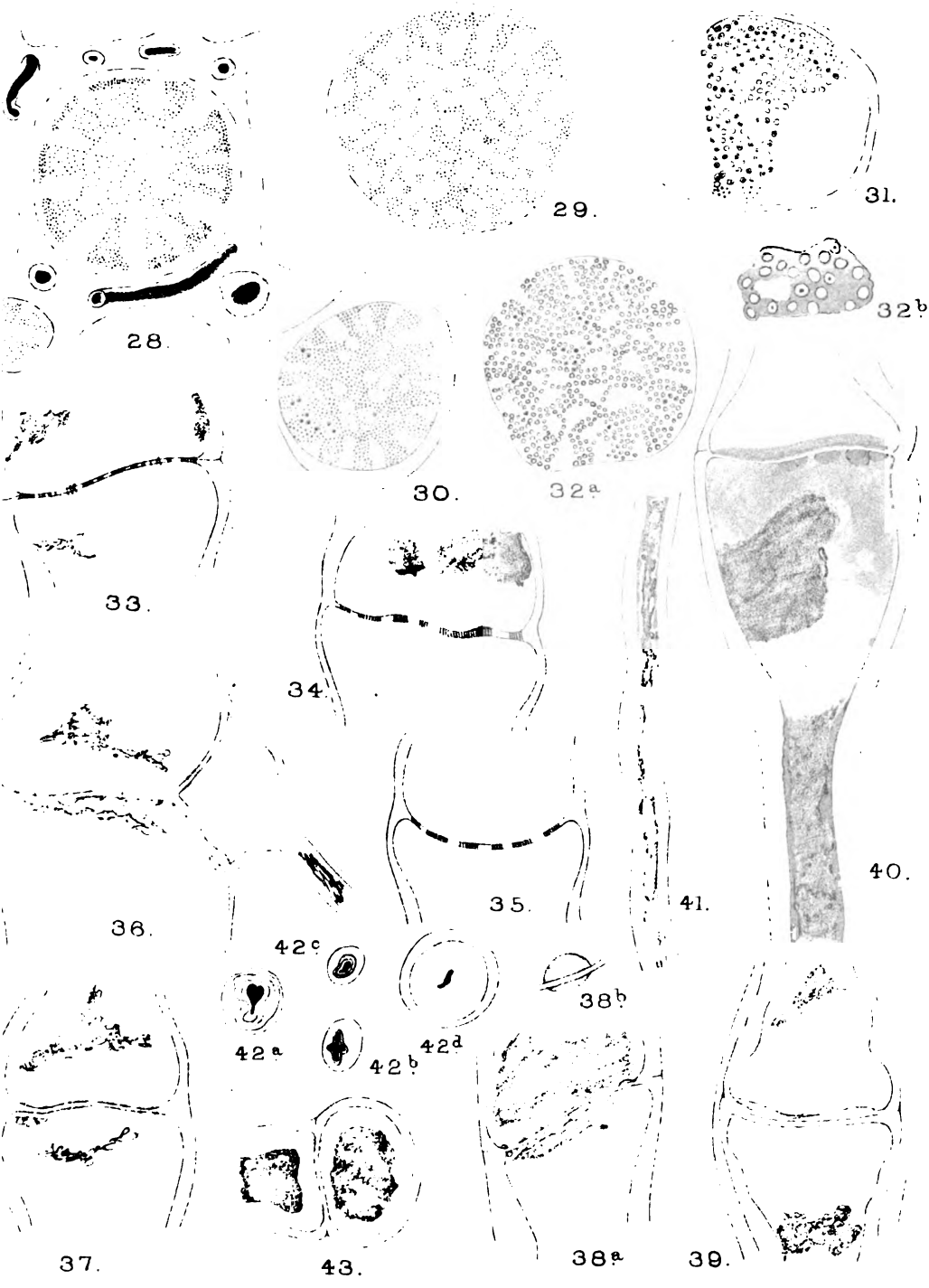


M.G SYKES – LAMINARIA SACCHARINA.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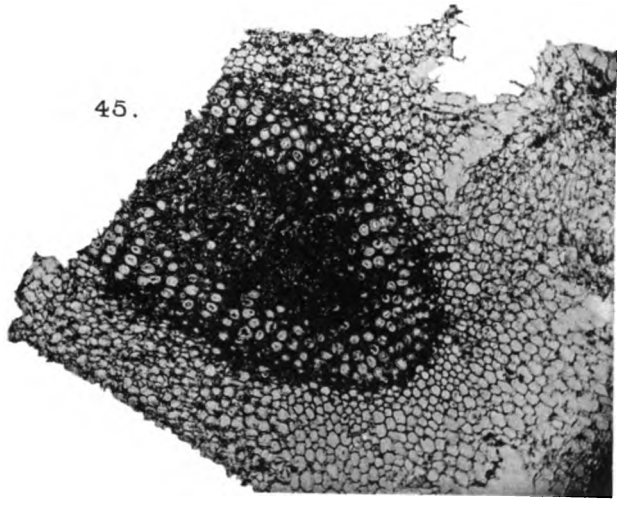

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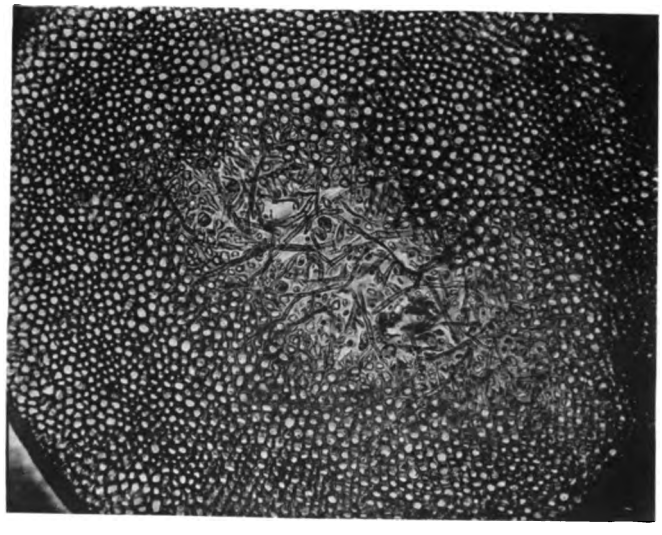

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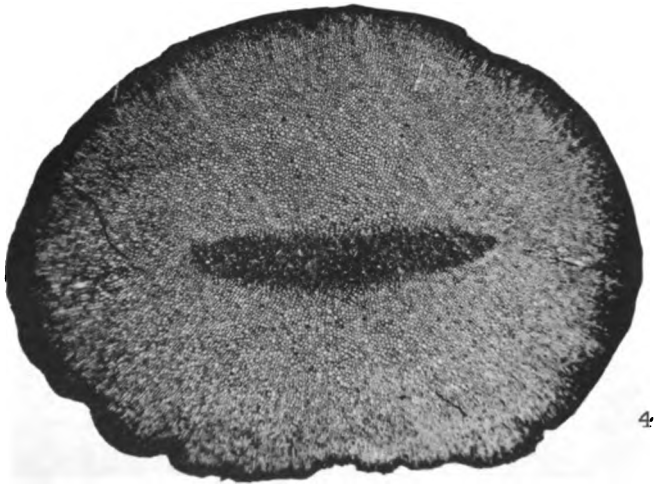

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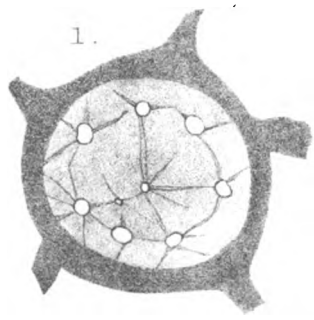
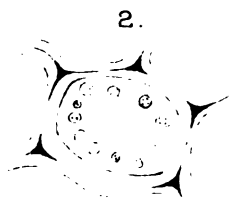
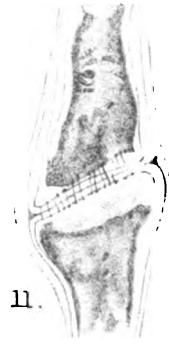
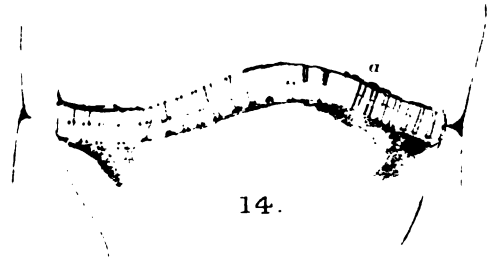
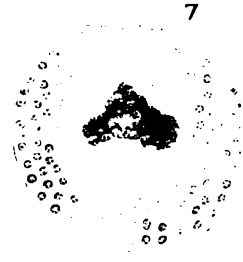
SYKES — MACROCYSTIS AND LAMINARIA.

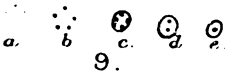


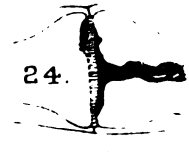


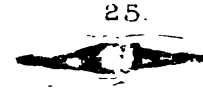


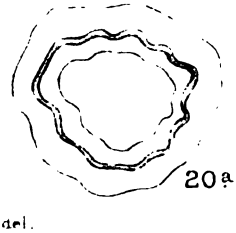


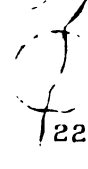


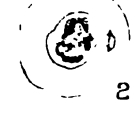












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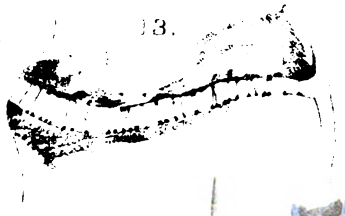

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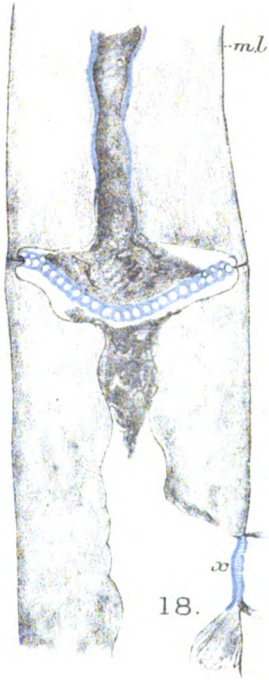

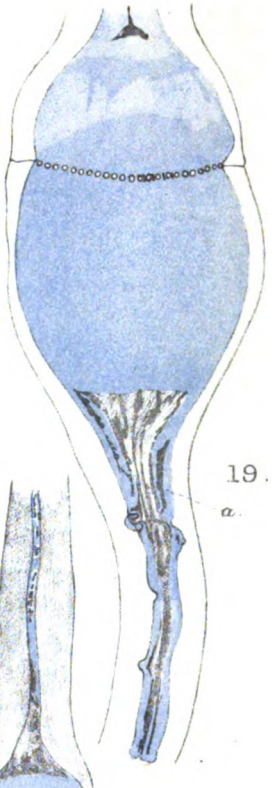

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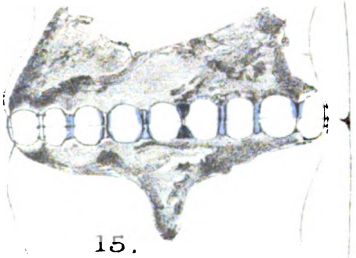

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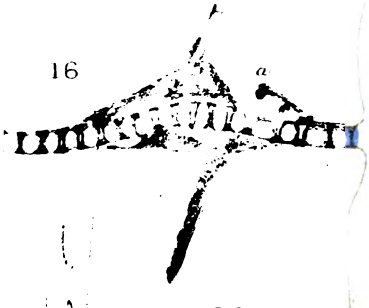

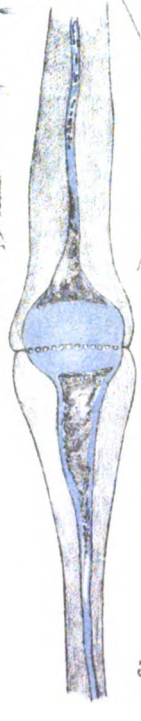

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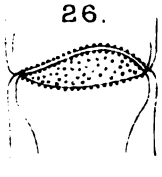

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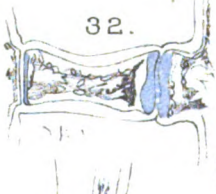

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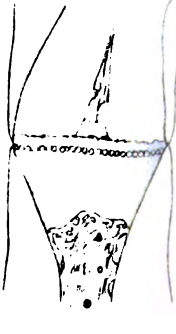

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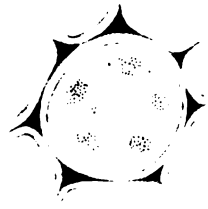
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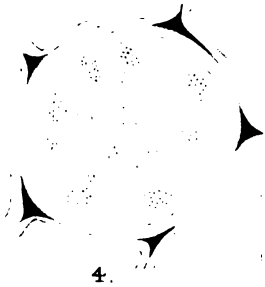
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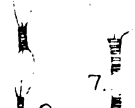
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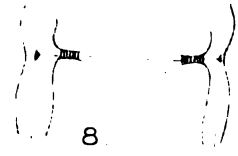
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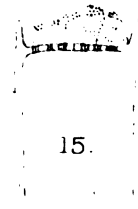
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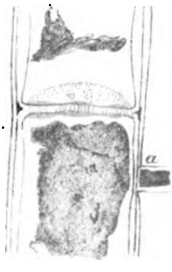
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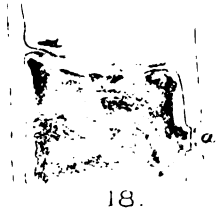
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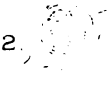
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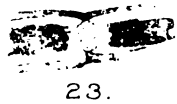
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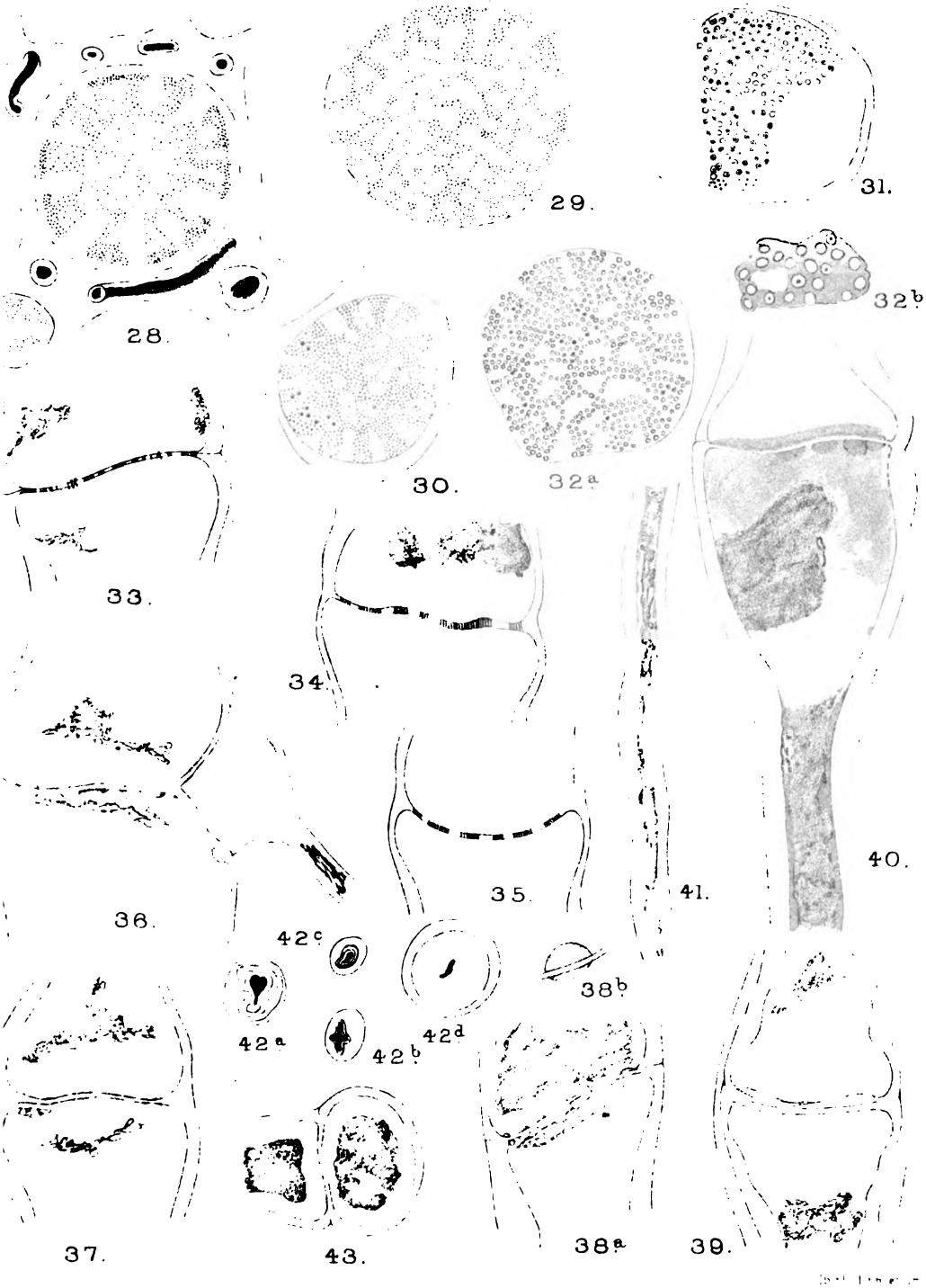
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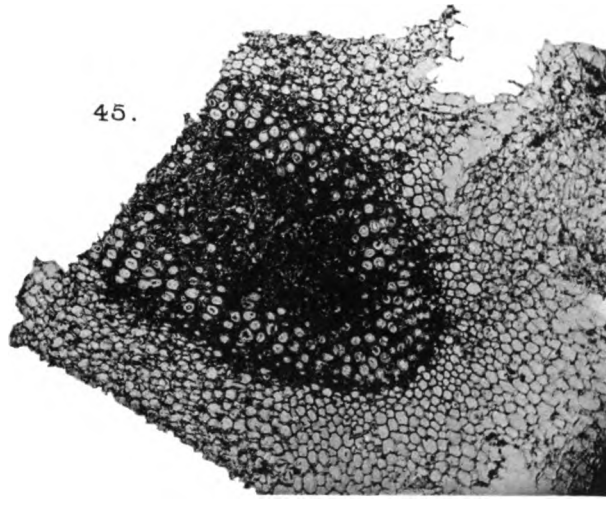

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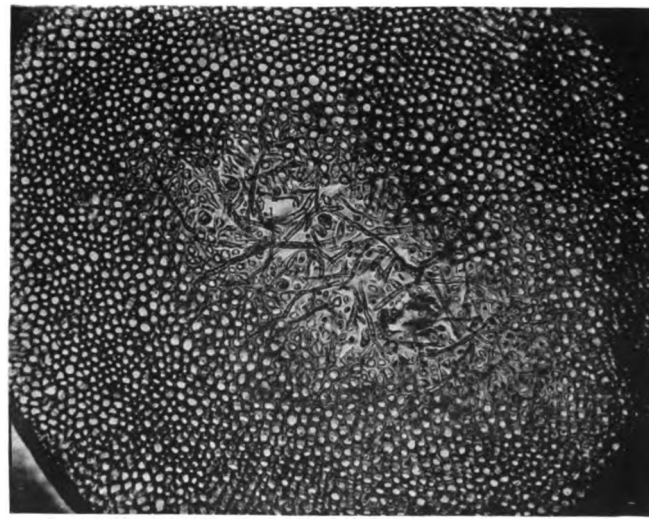

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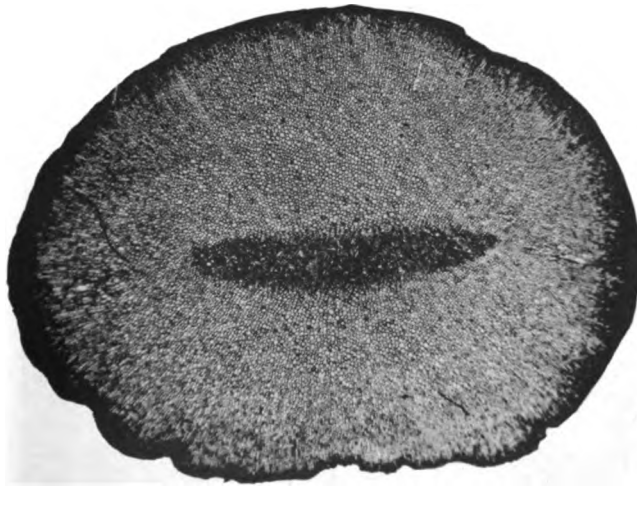

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SYKES — MACROCYSTIS AND LAMINARIA.

NOTES.

NOTE ON OPHIOGLOSSUM SIMPLEX, RIDLEY.—In the ANNALS OF BOTANY, vol. xviii, p. 205, I described from a single specimen, received through Prof. Groom from Mr. Ridley, the details of this Sumatran species. It differs from other Ophioglossaceae in the apparent absence of the sterile lamina, while the fertile spike is well developed. It was concluded, however, partly from the examination of the plant itself, partly from comparison, that the absence was not primitive, but the result of abortion. The species was referred to § *Ophioderma*, a section of the genus the species of which form a natural group anatomically distinct. Its three representatives were held to illustrate phases of decrease of the sterile lamina, from *O. pendulum*, where it is large, through *O. intermedium*, where it is small, to *O. simplex*, where the extreme condition is seen. This state may be attributed to the presence of mycorhiza, which makes nutrition of the large spike still possible in the dense wet forest in which it grows, notwithstanding that the usual assimilating organ is functionally non-existent. Campbell, however (Mosses and Ferns, second edition, p. 258), considers that *O. simplex* is not a reduced, but a primitive, form, and, in fact, 'the most primitive type of the genus yet discovered.'

Any further facts relating to so rare, and at the same time so debatable, a form, will have their interest. It was then a pleasure to hear from Prof. E. Rosenstock, of Gotha, that the plant had been again collected in Sumatra, in dense forest, on the Lalah river (Indragiri, West Coast). He was kind enough to send the specimens for examination, and consents to this note being published.

The specimens were evidently referable to *O. simplex* Ridley, but instead of the sterile leaf being entirely absent, as it appeared to be in Ridley's specimen which I examined, several of the elongated spikes bore, at a short distance below the fertile region, a more or less pronounced outgrowth, evidently representing a sterile lamina, while below that point there was a slightly increased width of the stalk. In dried specimens of such succulent organisms it is difficult to be certain of the natural form: there is, however, little room for doubt that Dr. Rosenstock's specimens were very like the plant figured by Sir William Hooker as *O. intermedium*, but with the sterile lamina very much smaller.

The fact that the minute sterile lamina thus exists in some, though perhaps not in all, specimens of *O. simplex* gives justification for the reduction hypothesis which I previously advanced, and links the species closer with *O. intermedium* and *O. pendulum*. In his latest contribution to the morphology of the Ophioglossaceae which deals with certain of their embryos (Ann. Jard. Bot. Buit., 1907) Professor Campbell has held as the most primitive forms of the genus those which diverge most widely from the type usual in other Pteridophytes. In holding *O. simplex* to be primitive he appears to take the same view in respect of a mature plant. It appears to me, however, to be a more probable view to hold those forms which are least divergent, such as the type of *O. vulgatum*, to be relatively primitive, while the three species of § *Ophioderma*

show varying degrees of specialization, for which the mycorhizic habit and the habitat give a reasonable explanation. Moreover, the anatomical fact that the leaf-trace in *O. pendulum* and *O. simplex* is composed of several strands at the base in place of one (l. c., p. 21) supports the opinion that they are not primitive types of the genus. Lastly, the fact that the vestigial lamina is clearly seen in some of Prof. Rosenstock's specimens would be less easily harmonized with the view of *O. simplex* as a primitive form than it would with the theory of reduction above expressed.

F. O. BOWER.

GLASGOW, *February*, 1908.

PRELIMINARY NOTE ON NUCLEAR DIVISION IN MNIUM HORNUM.—Nuclear division in the Mosses has up to the present time received but little attention. Beer[1] found four chromosomes in the dividing spore-mother-cells of *Funaria hygrometrica*, but stated later[2] that the number in this plant and in several other species, including *Mnium hornum*, was far greater. He also refers to the compound nucleoli in the spermatogenic cells of *Atrichum undulatum*.

The premeiotic divisions have been studied in the developing archesporium of *Mnium hornum*. The resting nucleus is made up of a fine homogeneous network and a single large centrally placed nucleolus. The latter contains almost the whole of the chromatin and by its persistence dominates the early stages of the division. At its first appearance the spireme consists of broad band-like masses of chromatin which are found especially at the periphery of the nucleus; by the further contraction of the chromatin a thin, thread-like spireme is formed. At this stage the nucleolus stains much less deeply, loses its sharp outline, and, a little later, breaks up and disappears. Twelve chromosomes are formed by the breaking up of the spireme, and these become arranged on the equator of the spindle showing the slender, hook-like form characteristic of the somatic divisions. The diaster originates in the usual manner by the splitting of the chromosomes, and during the anaphase and telophase the latter pass through changes resembling those described in the prophase.

A short period of rest elapses after the final division of the archesporial cells. The resting nucleus of the spore mother-cells shows the characteristic nucleolus and a somewhat coarser reticulum than that of the premeiotic cells. The closely coiled spireme arises by an increase of the chromatin in the nuclear reticulum. Contraction of the thread then takes place and results in a definite synapsis. On emerging, the thread thickens at the expense of the nucleolus which gradually disappears. The spireme divides up into six chromosomes which show the O- and the irregular X-forms characteristic of the reduction division. After separation the chromosomes pass outwards to form the daughter-nuclei. These do not enter into a completely resting condition, but quickly undergo the homotype division. This resembles the premeiotic divisions in its chief features. A full description of the divisions with figures will shortly be published.

M. WILSON.

ROYAL COLLEGE OF SCIENCE, S. KENSINGTON.

[1] New Phytologist, vol. ii, 1903, p. 166.

[2] On the Development of the Spores of *Riccia glauca*. Annals of Botany, vol. xx, 1906, p. 278, footnote.

A PRELIMINARY NOTE ON THE EMBRYO-SAC OF CERTAIN PENAEACEAE.—The Penaeaceae form a small and rather isolated group of shrubby xerophytes, of doubtful affinities, entirely confined to the south-western region of Cape Colony. The Order has not yet been thoroughly investigated; there are apparently five genera—*Sarcocolla*, *Penaea*, *Brachysiphon*, *Endonema*, *Geissoloma*, the last being placed by Engler in a separate Natural Order. Of these, I have investigated five species, representing the first three genera, and, as careful examination and comparison have revealed an exact similarity in the structure and development of their gametophytes and embryo, the following account may be taken as applicable to all three.

The macrospore mother-cell appears to form a row of three (?) macrospores, of which only the lowest persists, becoming the embryo-sac. This enlarges, and its nucleus divides to form four daughter-nuclei, which are arranged peripherally like the spores of a tetrad; an arrangement exactly the same as that described for the corresponding stage in *Peperomia*.[3] As the embryo-sac enlarges, a central vacuole is formed, and these four nuclei migrate to the parietal layer of protoplasm. Here, as in *Peperomia*, they show none of the polarity so characteristic of the usual type of Angiospermous embryo-sac, being irregularly distributed around the periphery of the embryo-sac. Usually, however, as the sac elongates two of the nuclei take up their position, one at or near each end, and the other two about its equator; but even this slight degree of polarity is often lacking, and the nuclei, or the cell-groups resulting from their division, may be arranged in any manner around the embryo-sac.

From this point the resemblance to *Peperomia* ceases, and here it may be noted that, while their embryos agree in the absence of a suspensor and in the formation of a spherical pro-embryo, the male gametophyte differs from that of any known Angiospermous type, while in *Peperomia* it is quite typical.

Each of these four nuclei now divides, and the two resultant daughter-nuclei again divide, so that four groups, each consisting of four nuclei, are formed, one group from each of the four original daughter-nuclei of the embryo-sac. Protoplasm now aggregates around three of the nuclei in each group, and a definite limiting layer —'Hautschicht'—appears about each of the cells thus formed, while the fourth nucleus remains free—so that in each group there are now three cells with definite membrane-boundaries, and one free nucleus. Either during or after the formation of the 'Hautschicht' round the cells, the free nucleus of each of the four groups migrates to the centre of the embryo-sac. There they meet, and gradually fuse to form the single large definitive nucleus.

While these nuclei are fusing, the four parietal cell-groups have each assumed the appearance of a typical egg-apparatus, and it would seem probable that this may be their true nature. The discussion of the evidence in support of this hypothesis is, however, better postponed until the phenomena accompanying fertilization have been more completely investigated.

[1] Annals of Botany, vol. xxi, p. 467; vol. xxii, p. 91.

[2] Lang, On the Sporogonium of *Notothylas*. Ann. of Bot., vol. xxi, April, 1907.

[3] Campbell, D. H., The Embryo-sac of *Peperomia*: Ann. of Bot., xv, pp. 103–17, 1901.
Johnson, D. S., On the Endosperm and Embryo of *Peperomia pellucida*. Bot. Gaz., xxx, pp. 1–11, 1900.

Whether anything corresponding to 'double fertilization' (a term here obviously inappropriate) occurs is not certain, though the evidence points to it. The definitive nucleus begins to divide before the fertilized egg. Its divisions are rapid and simultaneous, and, as might be expected from its multinuclear origin, the endosperm nuclei are large, with a great number of chromosomes and several nucleoli. They are distributed in the parietal layer of protoplasm, and walls appear only at a very late stage, forming a loose tissue in the lower part of the embryo-sac, which is entirely used up by the growing embryo.

These investigations were carried out in the Botanical Laboratory of the South African College, Cape Town, at the suggestion and under the supervision of Professor Pearson; they are being continued at the Botany School, Cambridge.

E. L. STEPHENS.

NEWNHAM COLLEGE, CAMBRIDGE.

THE EMBRYO-SAC OF PANDANUS. PRELIMINARY NOTE.—A preliminary study of the embryo-sac of *Pandanus* which I have recently made shows that it differs decidedly from the usual Angiospermous type, and to some extent resembles more nearly that of *Peperomia*. Two species of *Pandanus* have been examined—*P. odoratissimus* and *P. Artocarpus*—which agree very closely in the structure of the embryo-sac. Up to the second nuclear division in the young sac, *Pandanus* agrees with most of the Angiosperms, and at this stage the embryo-sac shows the usual two nuclei at the micropylar and chalazal ends. The two micropylar nuclei undergo no further division, but the chalazal nuclei divide until there may be twelve of these, and this seems to be the normal number, to judge from an examination of a number of the largest sacs that were studied. These always showed two nuclei at the micropylar end, surrounded by a relatively small amount of granular cytoplasm, while the large chalazal nuclei were scattered, apparently without any definite order, in a large mass of granular, more or less vacuolated, cytoplasm, which filled nearly one-third of the sac. The differentiation of antipodal cells was visible, and there was no certain evidence of nuclear fusions.

Whether any further differentiation of the embryo-sac structures occurs before fertilization remains to be seen.

DOUGLAS H. CAMPBELL.

STANFORD UNIVERSITY,
Feb., 1908.

SUPPLEMENTARY NOTE TO 'STUDIES ON SOME JAVANESE ANTHOCEROTACEAE'.[1]—Since this paper was written, Dr. Lang[2] has published an account of the sporogonium of *Notothylas Breutelii*, which closely agrees with that given in my paper for *N. javanicus*. The most marked difference is the greater development of sporogenous tissue from the endothecium in *N. Breutelii*.

DOUGLAS H. CAMPBELL.

STANFORD UNIVERSITY.

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ASSISTED BY OTHER BOTANISTS

London
HENRY FROWDE, AMEN CORNER, E.C.
Oxford
CLARENDON PRESS DEPOSITORY, 116 HIGH STREET
1908

Printed by HORACE HART, *at the* CLARENDON PRESS, *Oxford.*

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Sexual Cell Fusions and Vegetative Nuclear Divisions in the Rusts.

BY

EDGAR W. OLIVE.

With Plate XXII.

WE owe to the recent important discoveries of Blackman ('04) and Christman ('05) our knowledge as to the existence of a process of sexual reproduction which occurs at a certain stage in the development of the Rusts. Their accounts conflict somewhat, however, in respect to the mode of origin of the binucleated condition; hence the need of still further work on the cytology and minute structure of these forms. The following research explains to a certain extent some of the discordant results of the two accounts just mentioned, and at the same time extends our rather limited knowledge of the nuclear division in the vegetative cells. Further, the discovery of multinucleated cells at the base of certain aecidia, which may possibly be identical with the archicarps of De Bary, Massee, and Richards, obviously shows the urgent need of an investigation of the structure and development of further types of the aecidium-cup, before we shall be able to understand the possible connexion of this type of fructification with the simpler caeoma type, as well as its relationship to the fruit-bodies of other Fungi and Algae.

Earlier views as to the way the binucleated cells have arisen have been sufficiently reviewed in the papers of the writers just mentioned; hence a brief summary here will suffice. Rosen ('92) regarded the two nuclei in the aecidiospores as sister nuclei. The basal cell of the row of spores starts, according to him, with one large nucleus, which divides, and the upper of the two resulting daughter-nuclei at once divides again, this time at right angles to the axis of the elongated cell. The two nuclei thus formed in the apical end of the basal cell are then cut off by a cross wall from the one nucleus below, and thus form the beginning of the binucleated series. The uninucleated basal cell left below proceeds to divide again, and another binucleated cell follows as described.

Poirault and Raciborski ('95) apparently thought that the binucleated condition started in the sporidium, which they found sometimes contained two nuclei. They emphasized the fact, however, that the two nuclei which finally fuse in the teleutospore have no close relationship, since each has

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a distinct line of ancestors running far back in the life-history of the fungus.

Sappin-Trouffy ('96) also paid little attention to the origin of the binucleated condition, as he regarded the fusion of nuclei in the teleutospore as the most important stage in the sexual cycle. Poirault and Raciborski, as well as Maire, opposed this view, since they did not consider the fusion in the teleutospore as a sexual act.

Richards ('96) concluded that the binucleated cells in the Rusts had but little significance, since he thought 'that they are found in all of the other parts of the aecidia, in the hyphae, the pseudoparenchyma, and the peridium' (p. 260). Richards further considered the aecidium as more or less of a morphological unit, for he found at the base of the cup one, or occasionally more, large, sometimes multinucleated, cells which, according to him, give rise by budding to the basal cells at the bottom of each chain of spores. He was not able to trace the origin of these multinucleated cells.

Maire ('00), to whom we are indebted for first pointing out the existence of an alternation of generations in the Rusts, finds that in *Endophyllum* the vegetative hyphae are made up of uninucleated cells, to the very base of the aecidium. The basal hyphae, as well as the single nucleus in each, enlarge greatly. The binucleated condition is now brought about, according to him, by simple division of this one nucleus, unaccompanied by cell-division. The binucleated series starts, then, with sister-nuclei.

Blackman and Christman, notwithstanding their conflict in certain important details, agree in the crucial point, viz. that two uninucleated cells contribute to the initiation of the binucleated series. The main contention involves the character of the fusing cells—Blackman maintaining that the fertilization in the Rusts is an oosporic process, Christman, on the other hand, holding that it is zygosporic.

Blackman ('04), working with *Phragmidium violaceum*, describes the binucleated condition as arising from a 'vegetative fertilization', consisting of the migration of a smaller nucleus from an ordinary vegetative cell into a special 'fertile, or female' cell, containing a somewhat larger nucleus. Such a reduced sexual process has come about, according to him, through the replacement of the now functionless male cell, or spermatium, by an ordinary vegetative cell. A simpler 'internal' fertilization thus replaces the former 'external' fertilization, in which the spermatium functioned. He argues that the spermatia are male cells because of their resemblance to the spermatia of lichens, and because of their dense nuclei, and their small amount of cytoplasm, thus resembling strikingly male cells. He finds it impossible to accept the conidial nature of the spermatia, because of the difficulty in imagining a process of degeneration which brings about

a reduction of the cytoplasmic portion of the cell, but leaves the nuclei unchanged.

Each female reproductive organ, according to Blackman's conception, consists of a female cell below and a sterile cell above. He suggests that the sterile cell probably once pushed its way through the epidermis to the surface, and there functioned as a trichogyne, fusing with the spermatium. The vegetative nuclei migrate into the female cell either from the cell immediately below the female cell, in the same cell row, or from one of the smaller neighbouring cells at its base. The migration takes place, according to him, through a very small pore, that neither before nor after the passage of the nucleus could be distinguished. Whether any protoplasm passes over with the migrating nucleus Blackman could not determine.

Christman ('05), working with *Caeoma nitens*, *Uromyces Caladii*, and *Phragmidium speciosum*, described a process of true fertilization by the fusion of two cells, which he regarded as decidedly different from Blackman's method of nuclear migration. The two cells which fuse are of approximately equal size, as are also their nuclei. Both cells may, according to Christman, cut off sterile cells before becoming gametes. The fusing cells are figured as lying side by side, and a considerable portion of the walls in contact dissolve away, so that the product of the union is a distinct double cell, composed of the cytoplasm and nuclei from the two equal gametes. In harmony with this account, the row of spores formed from the growth of this fusion cell is regularly borne on two cells forming a double base. Blackman's basal cell, on the other hand, is really but one cell, with two nuclei. The latter contends that Christman's fusion occurs between two 'female cells'. Christman himself regards the fusion as rather of the nature of the conjugation of equal gametes, resulting in a non-resting zygospore.

Blackman and Fraser ('06), in their further studies on the sexuality of the Rusts, have investigated seven additional species, three for the development of the aecidia, three *micro*-forms, and one *lepto*-form. Nuclear migrations were found to be the means for the initiation of the binucleated condition in the aecidium cups of *Uromyces Poae* and *Puccinia Poarum*. In *Melampsora Rostrupi*, a caeoma form without pseudoperidium, the authors found indications of a conjugation of two equal cells, in the same manner as Christman described. They therefore now give equal rank to nuclear migration, or 'partial cell fusion', as they term it (p. 44), and fusion of two equal cells, as the mode of origin of the binucleated condition in the group.

Although convincing evidence was not obtained as to the method of transition from uninucleated to binucleated cells in the other four forms studied, the authors regard it as established that in the *lepto*-form, *Puccinia malvacearum*, the change to a conjugate condition takes place just before

the formation of teleutospores; while in the three *micro* species, the fertilization occurs at some earlier point in the life-history, without any immediate relation to the development of the teleutospores.

In a second paper, Christman ('07[1]) found that in the so-called 'primary uredo' form, *Phragmidium potentillae-canadensis*, the binucleated condition arises exactly as in the aecidium which he earlier described, by the fusion of two equal cells. He further emphasizes the interesting fact, also noted by Sappin-Trouffy, that the elongated stalk cells which bear the primary uredospores are plainly equivalent to the intercalary cells of the row of spores of the aecidium. Hence he argues that the primary uredo is morphologically an aecidium.

Still more recently, the same author (07[2]) has discussed the bearing of the facts, so far as known, on the general morphology of the various spore forms of the Rusts. He holds that the *micro-* and *lepto-*forms are the primitive Rusts and that the teleutospores are the primitive spores. The various other kinds of binucleated spores in the Rusts have been, therefore, gradually intercalated in the life-history. Christman holds that the binucleated 'basal cell' ('basidium' of certain older authors) is the morphological unit, since each subgeneration (bearing aecidio-, uredo-, or teleutospores) begins and closes with such a cell. The 'basal cell' produces its various kinds of spores in chains, or else singly, on long stalks, by a process of successive budding. He therefore concludes that the various kinds of sporophytic spores—aecidio-, uredo- (both primary and secondary), and teleutospores—are all homologous structures. The spermatia represent, in his opinion, the once functional asexual spores of the gametophyte; and he points out that they are quite similar in structure and general appearance to the other gametophytic spores of the Rusts—the sporidia—as well as to the pycnidia of certain Ascomycetes.

He finds also in *Puccinia Podophylli* frequent cases of the migration of nuclei in the teleutospore sorus from one sporophyte cell into another. Since no fertilization can be assumed in such instances, Christman interprets the phenomenon as purely pathological, perhaps due to the wound received at the time of fixation. He is therefore inclined to interpret Blackman's nuclear migrations in the vegetative cells at the base of the aecidia of *Puccinia Poarum*, as well as in other cases, as occurrences of a similar pathological nature.

The process of nuclear division in the Rusts is much better known for the nuclei in the promycelium than for the vegetative nuclei. Poirault and Raciborski ('95) studied the nuclear divisions in various types of cells of the group, and have obtained some very exceptional results. Each nucleus is regarded as containing but a single chromosome, which, during mitosis, splits longitudinally. During conjugate division, the two dividing nuclei form, in their opinion, but one spindle.

Sappin-Trouffy ('96) and Maire ('00), on the other hand, regard the two chromatin masses in each dividing nucleus, not as evidence of longitudinal splitting, but rather as showing that there are two chromosomes regularly present in the nuclei of the Rusts. While indirect division is described as the principal method of nuclear division, Sappin-Trouffy also finds cases of amitosis, especially in old mycelial cells.

Juel ('98), studying the promycelial divisions in *Coleosporium*, obtained results quite different from the authors mentioned above. His figures of nuclear division resemble closely those of other fungi. While not able to distinguish individual chromosomes, Juel thinks it quite probable that there are many.

Holden and Harper ('03) have recently shown that the process of division of the fusion nucleus of *Coleosporium* agrees in essential details with those described for higher plants. In the prophases, a spirem thread splits longitudinally. In the metaphases, polar radiations, centrosomes, spindle and chromosomes are all very sharply differentiated. The chromosome number is somewhere between six and ten. Conjugate division, in which the chromatin is represented as solid, dumbbell-shaped masses in all stages of division, is also figured by these authors; but they think it quite likely that poor fixation is responsible for the lack of differentiation.

Blackman ('04) has also investigated the subject of nuclear division, particularly in the promycelial cells of *Gymnosporangium*, and he has further compared with this type the divisions in the spermogonium, as well as the conjugate divisions in the sporophytic cells. The two divisions in the promycelium are regarded as reduced, or simple, forms of indirect division, while in the spermogonium and in the binucleated cells 'chromosome-formation is in complete abeyance, so that the division actually partakes of the nature of direct division (amitosis)' (p. 356).

In the promycelial divisions, according to Blackman, a well-defined spindle, centrosomes, and polar radiations are present. In the first division many chromosomes—at least ten—are formed; but in the second division the chromosomes appear to lose their individuality, and to form a solid mass. Blackman regards the spindle in the case of the second division of the promycelial nuclei, and apparently also in the case of the first, as formed free in the cytoplasm between the two portions of a divided extranuclear centrosome, and therefore as similar to the 'Centralspindel' of Hermann. His figures showing divisions in the spermogonium and in the binucleated cells merely repeat the poorly differentiated structures brought out by former authors.

Finally, Christman ('05, '07[1]) has figured the anaphases of the conjugate division, showing the two distinct spindles, the several chromosomes (the number was not determined), and a few short rays attached to each pole.

Material and Methods.

I am much indebted to Professor J. C. Arthur for the privilege of collecting rust material from his laboratory cultures, and for suggestions as to the most promising forms for study, as well as for other courtesies. I wish to acknowledge my obligations, also, to the Carnegie Institution of Washington for a grant which has made possible this collection. Of the forty species and more examined, perhaps the most favourable form for the study of sexual cell-fusions, as well as of nuclear division, was found to be *Triphragmium ulmariae* (Schum.) Link, on *Ulmaria rubra* Hill, a caeoma form, similar to the *Phragmidia* studied by Blackman and Christman. Cell-fusions are shown most clearly and unmistakably in *Triphragmium*, particularly after the walls of the hyphae have been specially stained; and the nuclear divisions in the spermogonium, as well as in both the gametophytic and sporophytic cells, show many details not hitherto described. Other species in which cell-fusions are described in this paper are: *Gymnoconia interstitialis* (Schlecht.) Lagerh. (*Caeoma nitens* S.) on *Rubus* sp.; *Phragmidium potentillae-canadensis* Diet. on *Potentilla canadensis*; and the *micro* form, *Puccinia transformans* E. & E. on *Tecoma stans* Juss.

Besides the fairly complete series of karyokinetic stages from *Triphragmium*, the following species have been utilized for isolated stages: *Uromyces Scirpi* (Cast.) Burr. (*Accidium Sii-latifolii* Wint.) on *Cicuta bulbifera*, *Uromyces Lilii* Clint. on *Lilium canadense* and *Puccinia Cirsii-lanccolati* Schroet. on *Carduus lanceolatus* L.

The material for the most part was fixed in various strengths of Flemming's Chromic-acetic-osmic acid mixtures. Some which was fixed in Juel's Zinc chloride-acetic-alcohol mixture proved to be particularly favourable for bringing out the cell-fusions, since the cell-walls seemed to color with especial tenacity after such fixation. With this method, absolutely convincing evidence was obtained as to conjugation pores, and as to the extent of absorption between fusing cells. Flemming's triple stain was used almost exclusively.

Sexual Cell Fusions.

Figs. 20–40, Pl. XXII, inclusive, drawn from four species of Rusts, show the method by means of which the binucleated condition suddenly arises, following a period of growth which produces only uninucleated cells. All four of the species here represented are of the diffuse caeoma type of aecidium. Although I have also found cases of fusions in the aecidium-cup type, certain peculiarities present in such forms, to be discussed later to a limited extent in connexion with nuclear division, necessitate further study before these more complicated conditions can be brought in line with the simpler caeomas.

As will be at once seen, all of the sexual fusions shown in Figs. 26–40 involve the conjugation of two uninucleated cells, through a pore of greater or less width. Further, since these figures are all similarly oriented, i. e. with the upper part of each figure pointed upward toward the top of the plate as toward the epidermis of the host, it is obvious that the fusing cells, at least so far as one can judge from sections, may be placed in almost any position with respect to each other. For example, the cells may be placed side by side, in the same plane, in much the same position as they are figured by Christman (Figs. 26, 27, 35, 36, 39, and 40). Perhaps even more frequently, in my experience, one of the fusing cells appears to lie somewhat below the other, as in Figs. 26, 28–34, 37, and 38, some of which suggest the conditions which Blackman figures (see, e. g. his Figs. 66–70).

Figs. 26–33 are all drawn from preparations of *Triphragmium ulmariae*. Fig. 26 represents a preparation in which two pairs of fusion-cells are shown side by side. Although at this stage in the development of the fungus sexual fusions may be found at many points along the diffuse sorus in the same section, one rarely meets with two such instances so near together, and at the same time properly oriented to serve for drawing. The boundaries of the cells below are lost in the mass of hyphae pushing up around them ; but the upper parts, showing the fusions, are quite clear. One nucleus from each pair is somewhat stretched out, apparently in the act of moving toward the other conjugating cell. In the instance at the left, however, the bud, which is put out after the fusion, is starting to grow in the opposite direction. One of the left pair in this case has a uninucleated cell above. Whether this is a 'sterile cell' or not, I am uncertain, since it stains quite darkly, and it does not appear to be in any way degenerating. In the other instance, however, at the right, the cell just under the epidermis is unmistakably a sterile cell, since it shows the characteristic signs of degeneration, both in its cytoplasm and in its nucleus. It is interesting to note that in this latter pair of cells the one is placed much lower than the other, and, further, the lower cell does not appear to have cut off any sterile cell at its tip. In the other pair at the left, the two cells at the time of fusing apparently had grown up to about the same level. The unusually long conjugation tube connecting the latter pair is another feature of especial interest.

In the next figure, 27, several interesting features are shown. In the first place, the conjugating cells are placed parallel to each other and at about the same level. Further, the right gamete has a degenerating sterile cell attached at its tip, whereas the left gamete has none. The point of contact of the two cells is apparently rather a small area ; hence the hole through which the fusion of the two protoplasts takes place is as yet comparatively small. The left nucleus is clearly moving over into the right half of the fusion-cell, so that it actually appears to have pushed into and slightly indented the other nucleus. It is quite possible that Blackman would

maintain that this is a case of nuclear migration. At the same time, it is undoubtedly a case of cell fusion, and quite similar to those instances described by Christman in which conjugation has occurred between two cells lying side by side.

In Fig. 28 the relation which the two fusing cells sustain to each other is somewhat uncertain. Although the lower cell appears to belong to the same hypha as the upper, I doubt that this is the correct interpretation, for careful focussing seems to show that the lower cell is from a distinct hypha which comes up obliquely toward the eye. It is important to note here again that the upper cell has attached to its tip, just under the epidermis of the host, a degenerating cell; and also that the budding growth has begun even before the lower nucleus has passed over into the companion cell. Many similar instances to the one figured show that the bud generally pushes out to one side of the sterile cell, instead of crushing directly into the latter.

Fig. 29 shows a much later development, in which the second bud has started off from the binucleated fusion cell. Even at this late stage, the interesting fact may be noted that two distinct cells enter into the composition of the fusion cell, or basal cell. The two nuclei lie, however, in the upper gamete. The remnant of the old wall, which formerly separated the two gametes, and through which the conjugation took place, is very sharply brought out in the figure, although it has apparently been almost completely absorbed. The younger bud at the right is cut obliquely lengthwise at its tip; hence its peculiar irregular shape, instead of the usual form with rounded end. An old dead sterile cell, not shown in the drawing, lies between the two buds. It probably once belonged to the upper of the two fused cells, but the rapid growth which followed the conjugation has displaced it from its original position. The larger bud in this case, although it has been cut off by a wall from the basal cell, does not yet constitute a spore, since a stalk will first be cut off below, to bear the oval spore at its tip. That the two nuclei of this bud are, in fact, in the prophases of conjugate division is indicated by the vacuolated condition of the nucleoli, and by the appearance of the chromatin. The same is true for the two nuclei below, but neither pair is adapted to show the minuter details of the process. Still later stages, in which the binucleated basal cell has successively budded off, in the manner indicated in Fig. 29, several spores, each borne on a slender binucleated stalk, are abundant in the same preparation. The method of formation of the primary uredospores of *Triphragmium*, therefore, agrees exactly with that described by Christman ('07[1]) for *Phragmidium potentillae-canadensis*, thus adding further confirmatory evidence to Christman's contention that the primary uredospores are morphologically equivalent to the catenulate aecidiospores.

Figs. 30–33 show still further variations in the relative positions of the

two conjugating cells of *Triphragmium.* In all these cases, one of the conjugants lies below the other, either immediately or else obliquely below. But, as has been intimated above, the evidence is not at all conclusive that in any case the two cells are derived from the same hypha. In fact, wherever positive evidence is present, it points out that the two cells are entirely distinct, and are not related as granddaughter cells of the same hypha, as Blackman thinks may be the case sometimes.

In Fig. 30 the partially absorbed partition wall between the two gametes appears to be peculiarly bent and contorted. The two nuclei now lie close together, and the fusion cell shows evidence of considerable growth. In Fig. 31 fusion has apparently just taken place, and the prophases of nuclear division have already begun. The sterile cell in this figure is particularly interesting. It has swollen up to an unusual size; further, the process of degeneration appears to have left but little of the cytoplasmic and nuclear contents, other than the more resistant nucleole.

Fig. 32 proves conclusively the statement made above, that the karyokinetic processes begin at once, on the very initiation of cell fusion, and some time before the gamete nuclei come to lie close together; since the migrating nucleus has cast out and left behind in the lower gamete its nucleole. As will be seen later, this throwing out of the nucleole is one of the first indications of mitosis.

Figs. 33 and 34 show conditions of particular interest, the significance of which will be discussed later, since in both instances nuclei from a lower cell are apparently migrating into an upper cell. Fig. 33 is from *Triphragmium,* while Fig. 34 is from a preparation of *Gymnoconia interstitialis.* These figures are both quite comparable to Blackman's Figs. 66 and 68. In neither of my figures, however, is there such a tiny pore present as must be assumed to be the case in Blackman's Figs. 67, 69, and 70, where the migrating nuclei are drawn out to narrow threads in the process. Figs. 33 and 34 simply represent cases in which the fusion of the two cells is initiated by the beginning of the passage of the protoplast through a very small pore. In Fig. 33 the nucleus has just begun to pass through from the lower into the upper cell. Both gametes are obviously from distinct hyphae in this instance, as are also those of Fig. 34. In the latter figure, fully half of the long, stretched-out nucleus has passed through the narrow opening into the upper cell, and now partially overlies the nucleus of the upper cell.

Figs. 35 and 36 represent also cell fusions in *Gymnoconia*; both are of a similar type, in that the fusion occurs between two cells placed adjacent and parallel. These figures recall Christman's ('05) Fig. 5, in which one gamete lies slightly higher than the other. It is a highly interesting fact, and one which will call for comment later, that Figs. 34 and 36 are drawn from the same section, and are situated only a short distance from each other in the preparation. Here appears to be an instance in which

a Blackman 'nuclear migration' occurs adjacent to a case of 'cell fusion' similar to that described by Christman.

Figs. 35 and 36, as indicated above, are quite similar to each other as to their method of fusion. In Fig. 35, however, the walls below, between the two gametes, are shown as one; while in the other figure the walls are quite distinct from each other. In both cases, further, a concentration of finely granular protoplasm is evident in the upper part of the fused cells, where the protoplasts have mixed in, and on either side of, the conjugation pore. In Fig. 35, finally, each nucleus shows a small, deeply stained, dumbbell-shaped body—the dividing centrosome—whose significance will be discussed in some detail later.

Fig. 37, from a preparation of *Phragmidium potentillae-canadensis*, although poorly stained as to the nuclei, shows very clearly the beginning of the formation of the conjugation pore. Here again the relation of the two cells to each other is in doubt. Although they appear to be in the same cell row, the lower cell may just as well be from one of the obliquely lying hyphae which come up from below at varying angles. In this figure, a granular body, resembling a nucleole, lies in the pore connecting the two cells. In other cases I have found unmistakable cases of nucleoles thus thrown out at the beginning of the nuclear division, which starts with the earliest indication of cell fusion. But in this instance vacuolated nucleoles are apparently still present in both poorly-defined nuclei, so that it seems likely that the granular body is merely a cytoplasmic inclusion.

Fig. 38 is from the same preparation of *Phragmidium potentillae-canadensis* as is Fig. 37. Such poorly fixed and stained preparations were thus selected because the remnant of the partition wall between the two fused cells stands out in each case with great distinctness. The leaf in this instance was fixed with Juel's fixative and stained with Flemming's triple stain, as was also the material from which Figs. 28–32 were drawn.

Figs. 39 and 40 represent fusing cells of the *micro*-form, *Puccinia transformans*. In the first-named figure, the nuclei, except for their bright red nucleoles, are not well defined. Fusion has apparently taken place in this instance between the tips of two parallel hyphae. In Fig. 40, on the other hand, an end cell of one hypha (which apparently comes in obliquely from below) has fused with the penultimate cell of another. The uninucleated character of the gametophytic cells is also well shown in the latter figure. While it is not my present purpose to discuss this *micro*-form in detail, I may say in passing that the fusion is followed at once by a growth resulting in teleutospores, each borne on a stalk consisting, at least in some instances, of about three binucleated cells. The sporophytic development consists, therefore, of a few binucleated cells only. This case is possibly similar to that of *Puccinia malvacearum*, in which Blackman finds that the change from a uninucleated to a binucleated condition occurs at the base of

the teleutospore sorus, although he is quite at a loss to explain how the transition has come about.

Vegetative Nuclear Division.

As indicated in the review of the present status of our knowledge concerning the nuclear division in the Rusts, the subject greatly needs further investigation. *Triphragmium ulmariae* proved to be an especially favourable form, since the large nuclei show in their division stages many details not hitherto described. The caeoma-like aecidium of the thistle Rust as well as some other forms were also found to possess comparatively large nuclei, but the mitotic figures were not so clear in these species.

Fig. 1 shows one of the exceptionally large nuclei characteristic of the spermogonial hyphae of *Triphragmium.* A comparison of Fig. 1 with Figs. 12, 26, and 27, which are drawn to the same scale, will serve to emphasize the difference in size between the nuclei of the spermogonium and those of the germ cells of the young sporophyte. In Fig. 1 will be seen a small nucleole, a lightly-staining chromatin network, and, in particular, two small, deeply-staining bodies on the nuclear membrane, each surrounded by a small aggregation of a less deeply-staining archoplasm-like substance. These two centrosomes, as I shall term them, now lie close together, and each has attached to it a well-defined chromatic filament. I am inclined to think that this figure represents an early prophase condition, in which the centres have just undergone division; but there is another possible view, which will be explained in some detail later, viz. that the two centres have been carried over as a double centre from the last previous division.

Fig. 2 is much easier to interpret. The two distinct centres shown in this figure have obviously migrated apart, until they now lie at quite a distance from each other, and they are joined together by a long, slender strand. Judging from careful focussing, the strand apparently lies uppermost, on the surface of the nucleus, which still contains some nuclear sap and is still enclosed in a nuclear membrane. In this figure it is clear that the deeply-staining chromatin, particularly in the basal portion, is attached partly to the centres, and, at least apparently, partly to the narrow strand itself. As will perhaps be more clearly seen in figures of conjugate division, to be explained later, this strand connecting the diverging centres undoubtedly corresponds to the 'Centralspindel' of Hermann ('91).

In the next figure, 3, which is somewhat more highly magnified, the central spindle now extends to the extreme ends of the elongated nuclear figure. Apparently the nuclear membrane has not yet completely broken down, as some sap still seems to be present in the lower part of the nucleus. The nuclear content in the upper half of the figure, in the spermatial bud, is obscure. It appears to be composed mainly of a thready substance. The chromatin obviously lies at one side of the spindle figure, and apparently

almost entirely in the lower, more swollen part of the nucleus. Whether this nucleus has yet passed through the equatorial-plate stage I am not able to state positively, as this stage is somewhat obscure for the spermogonial nuclei of *Triphragmium*. This phase is more clearly brought out in the later sporophytic divisions.

Fig. 4 shows a somewhat later stage of division in the spermatial filament of *Triphragmium*. This seems to be an early anaphase, since some of the chromatin still occupies a more or less central position, while other chromatin material has already been drawn closer to the poles. The central spindle is here shown to be a rather thick, achromatic strand, situated a little to one side of the axis of the figure, while the mantle fibres are also more or less clearly defined, having one end attached to the poles, and extending thence along the axis as granular filaments, which appear to be mostly chromatic in their staining reactions. An aster is shown at the lower pole, and also at the lower pole of Fig. 3.

Fig. 5, from the spermogonium of *Uromyces Lilii*, although somewhat ill-defined and difficult of interpretation, apparently represents a stage next in order of development. The long axial strand is probably the central spindle, while the mantle fibres, particularly at the lower pole, suggest a late anaphase. It is of interest to observe in this figure that, although mitosis is somewhat far advanced, the dividing nucleus has not yet passed into the end of the filament, where the spermatial bud is already well formed. Herein this form on *Lilium* differs markedly from *Triphragmium*, in which the nucleus, when in quite an early stage of mitosis, moves upward into the very tip of the budding spermatium (see Figs. 2, 3, 4, 6).

A late anaphase of the spermogonial nuclei of *Triphragmium* is represented in Fig. 6. Such a figure is perhaps most often met with, presumably because its sharply-defined poles so easily catch the eye. Only a few obscure filaments now remain of the central spindle. But the mantle fibres, on the other hand, are at this stage so conspicuous that they can be quite easily and accurately counted. In Fig. 6*a*, for example, a daughter-nucleus in anaphase condition is drawn, in which the filaments attached to the poles are seen to number eight. Eight indicates also, in my opinion, the chromosome number for this form.

This last-mentioned figure (Fig. 6*a*), which is drawn with a very high magnification, shows most clearly a peculiar phenomenon. The centre is here plainly a double structure, and each half has four clearly-defined strands attached to it. The double nature of the centrosome is also shown at both poles of Fig. 6, and at the lower pole of Fig. 3. Two possible explanations of this phenomenon are apparent. The doubling has obviously arisen either from a premature division of the centre preparatory to the next division; or else it results from the close proximity in the same nucleus of the chromatin contents of the maternal and paternal nuclei,

which fused in the teleutospore, a short time before this stage. The great theoretic importance of the latter theory is sufficiently obvious, and will be discussed to a limited extent later in the general part of this paper. No signs of the doubling of the centrosome are to be noticed in the newly reconstructed spermatium nucleus drawn in Fig. 7, nor in Fig. 8, which represents a highly-magnified sporophytic nucleus from the young aecidium of *Puccinia Cirsii-lanceolati.* So far as can be seen, the centres in each of these figures are single structures. It is at once obvious, particularly in Fig. 8, that the chromatin is centred on this structure. One end of each of the chromatic filaments is connected with the centre, while the other extends into the nuclear cavity, thus resulting in a polarized condition of the nucleus, similar to that described for certain mildews by Harper ('05).

Fig. 9 is from the spermogonium of *Uromyces Scirpi*, and represents an equatorial-plate stage. The ill-defined chromatin is massed somewhat excentrically in the equatorial region of the dense spindle. Centrosomes are somewhat obscure in this instance; possibly the broad structure at the lower pole is to be regarded as a sort of pole-plate, or disk-shaped centrosome.

The next three figures show gametophytic nuclei, as is true also of all the figures of nuclei so far described, except Fig. 8. Figs. 1–7 and 9, however, are all drawn from spermogonia; while Figs. 10, 10*a*, and 11 are from the vegetative hyphae which finally produce the gametes. All three of the latter drawings show the anaphases of the division, by means of which the so-called 'sterile cell' is cut off. Figs. 10 and 10*a* are from *Triphragmium*, and Fig. 11 is from one of the hyphae from the base of the aecidium-cup of *Uromyces Scirpi.* In Fig. 10, reconstruction is in process. Above, the centre is obviously double, and about four strands are attached to each half-centre, just as was described for Fig. 6*a*. The daughter-nucleus below, in Fig. 10, shows an obliquely polar view, so that about eight chromatic filaments can be counted, radiating from the pole. A new nucleole is in process of formation within this latter nucleus. In Fig. 10*a* the mitotic figure is apparently rather poorly defined, as is also the late anaphase shown in Fig. 11.

Figs. 12–22 show a fairly complete series of stages of the mitoses which take place in the sporophytic cells containing the so-called 'conjugate nuclei'. While considerable growth takes place in the gametophytic hyphae which have just been considered, a greatly increased stimulus to growth is apparent at once on the initiation of the conjugate, or binucleated, condition, through the fusion of two cells. The first step in the rejuvenating sexual process is thus the sudden transition from uninucleated cells to binucleated.

As has been described above, when the cytoplasm of two cells begins to fuse, through the gradual absorption of the wall which separates them,

the two nuclei begin at once to divide, even before they come to lie close together in the fusion cell. This is proved, as I have already suggested, by the immediate formation of vacuoles in the nucleoles, and by numerous instances in which the nucleoles are cast out into the cytoplasm before the two gamete nuclei have approached each other (as, e.g., in Fig. 32). Such nuclei as are shown in Fig. 35 also illustrate this point. The dumbbell-shaped centrosomes in this instance are obviously in a state of division, although cell fusion has only recently taken place, and the two nuclei still lie in the bases of their respective gametes.

Fig. 12 represents a condition just following cell fusion, although, in this instance, the origin is not shown. The long, budding growth contains the two conjugate nuclei, each of which has begun the prophases of division. Here again, as in Fig. 35, are seen two dumbbell-shaped centrosomes. Each centrosome appears to be contained within the nucleus; but careful focussing inclines one rather to the belief that it is not an intranuclear structure, but that it lies instead on the surface of the nuclear membrane. Figs. 1 and 2 show this point more clearly, for there can be no doubt in these instances that the centres, as well as the central spindle which is formed between them, lie, at least for a time, on the nuclear membrane. Further, the connecting isthmus of each dumbbell-shaped structure in Fig. 12 corresponds, in my opinion, to the long, slender strand, or central spindle, extending between the centres in Fig. 2.

The nuclear membrane soon breaks down, and the nucleole is thus cast out into the cytoplasm, as Figs. 13–15 show. Apparently this phenomenon is about to take place in one of the nuclei of each of the following figures, 12, 29, 32, and 34. The central spindle early becomes a strongly-developed structure, apparently of a densely filamentous nature, which stretches between the two diverging centrosomes. The chromatin at this early stage forms an ill-defined, more or less thready mass at one side of the central spindle, much as Hermann and others figured for the nuclei of the Salamander and other animal cells (Figs. 13–15, 24).

The central spindle is frequently at first somewhat curved, or bowed in, on the side on which the chromatin is massed, as is shown for certain spindles in Figs. 14 and 24. Further, during the divisions just following conjugation, the two spindles are often variously oriented with respect to each other. In Fig. 14, for example, the two spindles are placed almost at right angles to each other. In Fig. 13, while the mitotic figures are about parallel to each other, they are twisted about so as to lie across the long axis of the cell. In Figs. 15, 17, 18, and 20 the more common position is shown, the two spindles lying parallel to each other and in the long axis of the cells.

An early prophase of conjugate division is shown in Fig. 13. The nucleoles are somewhat near the central spindles, which are at this stage comparatively short. The poles of the spindles are each terminated by

broad, plate-like centrosomes. A somewhat later stage is shown in Fig. 41. The nucleoles now lie some distance from their respective spindles. The left nucleus in this figure is particularly well adapted to show the relation which the dense central spindle sustains to the chromatic mass aggregated on its curved side. Mantle fibres are here not well differentiated; but it seems quite likely that they are represented by the few dense filamentous structures which curve from the poles out into the adjacent mass of chromatin.

Fig. 15 shows a somewhat similar stage of conjugate division. The two nucleoles are quite remote from their nuclei, and they show as yet no particular signs of disintegration. The double base of this figure suggests that this is a 'fusion cell', but there are no remnants of a partition wall left to show how such a fusion took place.

While unsatisfactory as to the chromatin, Fig. 16 shows with remarkable clearness the sharply-delimited spindles. There can be no doubt that here the two spindles are separate and distinct structures, and that they do not fuse into one during division, as was claimed for conjugate divisions by Poirault and Raciborski. The chromatin in this instance has apparently been drawn almost entirely into the central spindle, and it is now in early anaphase condition. The two nucleoles lie near the equator of their respective spindles and are in an advanced stage of dissolution, as is indicated by the large vacuole in each.

While it is sometimes difficult to distinguish the central spindle in the late anaphases, the mantle fibres, on the other hand, stand out sharply. A good part of the fibrous structures which appear between the receding centres in Fig. 17 probably should be referred to the mantle fibres, since they are chromatic in their staining reactions. In Fig. 18, also, the conspicuous fibres are probably mantle fibres, and the few dim structures, which still persist between the daughter-nuclei, doubtless represent what is left of the central spindle. In many other instances, however, these late stages are characterized by the persistence of a quite conspicuous central spindle. The attenuated structures in Figs. 20 and 21, for example, which connect the receding daughter-nuclei are plainly central spindles, and the mantle fibre portion in both cases has contracted almost to the poles.

The frequent presence of asters at the poles during these later stages (Figs. 17–19) undoubtedly indicates a totally different sort of polar activity than that which characterizes the earlier stages, since in the latter they are not noticeable see Figs. (13–16).

The reconstruction of the daughter-nuclei in these forms has already been described for the gametophytic hyphae, and the process in the binucleated cells is essentially similar. Fig. 19 illustrates quite clearly the phenomenon. The chromatin of each nucleus lies in a clear space, and the strands are arranged with some regularity. One end of each strand is still attached to the centre, while the other apparently ends free in the

nuclear sap or else anastomoses with other strands. Although a considerable amount of nuclear sap seems to have been already formed in this instance, the nuclear membrane cannot yet be distinguished. As in the cases described above for the gametophytic hyphae, the chromatin strands which radiate from each centre are plainly constant in number, being about eight in each of the three nuclei shown. The fourth and missing nucleus in this cell lies immediately below the one shown in the tip of the hypha.

The large size of the nuclei of *Triphragmium* is emphasized by a comparison of Fig. 19 with Figs. 20 and 21, which represent the close of the conjugate divisions in the aecidium of *Uromyces Scirpi*. All three of these figures are drawn to the same scale. The latter are too small to show the details of the telophases; but the centres, chromatin strands, and central spindles can be made out in properly-stained preparations, and these features will be seen to correspond in every way with those described for the larger and more favourable nuclei.

It will be remembered that Richards ('96) found at the base of the aecidium-cup of the Rust on *Ranunculus septentrionalis* a single large 'fertile hypha' which contained many nuclei and which budded out to form finally the basal cells. Massee ('88) had previously figured a large several-nucleated cell which he termed the 'oogonium', at the base of the aecidium-cup on *Ranunculus Ficaria*. Blackman and Fraser ('03) also show drawings of *Puccinia Poarum* (Figs. 15 and 16) in which three and four nuclei are seen in division; but they regard such cases as abnormal.

In the species occurring on *Cicuta* (*Uromyces Scirpi*), as well as in six or eight other species, I have found multinucleated cells, sometimes only one, or again many, generally scattered more or less irregularly at the base of the aecidium-cup. Fig. 21 illustrates one of these cells, which contains four nuclei in an advanced state of division. The parallelism of the four spindles in this case is striking, only one of the nuclei being placed at a slight angle to the others. Notwithstanding this slight displacement, the two pairs of nuclear figures may be still regarded as maintaining their conjugate arrangement. In the later development of the fungus, when two nuclei only occupy the cell, the close proximity and parallelism of the two figures appears to be maintained with the greatest regularity.

Another four-nucleated cell is shown in the next figure (22), from the base of the aecidium on the thistle. Nuclear division has apparently just been completed, since the nuclei show obvious signs of undergoing reconstruction. Three of the nuclei, for example, still have the chromatin massed about their centres.

Much more striking cases of multinucleated cells occur in Figs. 23, 24, and 25, two of which (23 and 25) are from the base of the aecidium on the thistle. Besides the nine poorly differentiated nuclei shown in Fig. 23, three possessing the same characteristics and undoubtedly belonging to the

same cell are to be found in the next section of the series. Other cells round about the one here illustrated have resting nuclei which seem to be exceedingly well fixed (see, e. g. Fig. 25, in which fifteen such nuclei are shown in one cell), so that the peculiar appearance in this one cell can hardly be said to be due to poor fixation. Although I am not prepared to explain all the details of the mitosis for this form—*Puccinia Cirsii-lanceolati*—I am fairly certain that such stages as are here represented are early prophases. We have then presented in this instance the peculiar phenomenon of twelve nuclei in the one cell undergoing division at the same time. Further, the variously irregular positions assumed by the figures suggest that the paired, or conjugate, arrangement seen in the binucleated cells is here not at all maintained.

A still more convincing case is shown in Fig. 24, from the basal region of the aecidium on *Cicuta*, in which the six nuclei here drawn (as well as three additional ones which lie in a lower plane) show clearly the prophases of division, as well as the fact that the spindles maintain no definite paired relation to each other. The various central spindles are oriented in such a heterogeneous fashion with respect to each other that it is difficult to imagine how, in these multiple divisions, there can be any maintenance of a conjugate relation. The principal point to be noted in this place, however, is the fact that where such multinucleated cells occur, the tendency is for the nuclei to divide simultaneously. But that this is not universal in such conditions is indicated by some apparent exceptions which I have found. While it is not the purpose of this paper to discuss the important questions which arise as to the origin of these multinucleated cells, and as to the part they take in the development of the aecidia, I wish merely to add the suggestion that the lack of evident pairing of the nuclei is probably comparable to the somewhat irregular relation seen in the young fusion cells of *Triphragmium* (Fig. 14), where the paired spindles may at first be variously oriented with respect to each other. It is, of course, possible that we have here multinucleated gametophytic cells, in which we should expect no pairing of the nuclei. But, although convincing evidence is as yet lacking, I am inclined to regard such cells as sporophytic in their origin, and as resulting from the greatly stimulated growth which follows sexual cell fusions.

General Discussion.

The conjugations by means of which the cells of the Rusts become changed from a uninucleated to a binucleated condition are certainly not to be associated with the simple anastomoses or fusions for nutritive purposes such as occur in the hyphae of many fungi. As Maire, Blackman, and Christman agree, this step in the Rusts marks the beginning of the binucleated condition of the sporophyte generation. The uninucleated

stage, on the other hand, from the sporidia, or basidiospores, up to the base of the aecidium constitutes the gametophyte generation. Besides the basidiospores, this stage bears only one kind of uninucleated spore-like bodies—the so-called spermatia—which Christman regards as the now functionless gametophytic pycnidia; whereas the sporophyte stage may bear from one to several kinds of binucleated spores. The process of sexual cell fusion which initiates the binucleated condition gives the stimulus of 'fertilization', or the first essential step of the sexual cycle. This is followed, either shortly (in the *lepto-* and *micro-*forms) or after a more or less prolonged period of development (in the *eu-*, *brachy-*, and *hemi-*forms), by the other steps of the sexual process, i.e. nuclear fusion, and, according to Blackman, chromatin fusion.

The main question here confronting us involves, therefore, the method of fertilization—as to whether 'a female cell is fertilized by the nucleus of an ordinary vegetative cell', as Blackman maintains; or whether the two fusing cells are equal gametes, as Christman holds. The one evidently interprets the process as tending to the oosporic type and toward the Red Algae; the other holds that two equal gametes fuse to form a non-resting zygospore.

Although in Figs. 28, 31, and 32 the lower cell appears to be somewhat smaller than the upper gamete, it is sufficiently obvious that such sections as are here represented will not enable one to judge certainly as to the shape and comparative size of the two fusing cells. In his first paper ('04), Blackman makes a strong point of his observation that the lower cell is smaller than the upper cell; and, further, that even the two nuclei in the young fusion cell are unequal in size, remarking that the smaller nucleus is denser and that it shows no nucleole or only a small one. In each of the Figs. 31–33, 36, and 40, one of the two nuclei likewise appears to be smaller than the other; although the nucleoles are not lacking in these cases, nor are the smaller nuclei conspicuously denser than the others. Some instances of fusing cells which I have observed, in which one of the nuclei appears to be smaller than the other, are undoubtedly to be explained as due simply to the unequal cutting in two of the smaller nucleus; or it may be that an end view of an elongated nucleus is presented. One nucleus, it is true, may, during the passage from one cell to another, be somewhat denser than the other (Figs. 33 and 34); but, in my opinion, there are no differences in shape or size, either between the two fusing cells or between their nuclei, which cannot reasonably be explained as due to sectioning or to greater or less rotundity of the nuclei.

It is clear, therefore, to my mind, that Christman's contention as to the equality of the two fusing protoplasts will hold, at least for the four species herein considered. Hence Blackman's interpretation of one of the cells as a differentiated 'fertile, or female' will not apply in these cases. But

the latter's conception of fertilization by means of 'nuclear migration' has, in my opinion, some basis in fact, since, during the earlier stages of cell fusion, one of the two nuclei may undoubtedly sometimes be seen to pass through a very narrow opening into the adjoining gamete (Figs. 33 and 34). But a multitude of other cases in which a broad fusion pore is formed (Figs. 26–33, 37–40) proves that the nuclei are not alone involved, and that the cytoplasm of the two cells also fuses into one mass. As indicated above, the occurrence of both the Blackman and Christman types of fusion (Figs. 34 and 36) near each other in the same preparation shows conclusively that appearances which suggest a 'nuclear migration' through a narrow opening may occur adjacent to a clear case of cell fusion in which a broad conjugation pore has been formed. Since the term 'nuclear migration' has been recently used extensively for undoubted pathological cases in which nuclei are found passing through minute pores in the partition wall; and, further, since Christman ('07 [2]) has shown that similar pathological migrations may sometimes occur between sporophytic cells in *Puccinia Podophylli*, it seems desirable not to employ the expression 'nuclear migration' for those cases in which, during the earlier part of the sexual cell fusions, the nuclei are seen to pass through narrow openings into the adjoining gametes. Such true sexual fusions as are shown in Figs. 33 and 34, as well as in Blackman's Figs. 66 and 68, certainly resemble strikingly the pathological nuclear migrations figured by Miehe ('01) and Christman ('07 [2]). But the subsequent history of the binucleated series of cells thus sometimes initiated in the Rusts undoubtedly excludes any interpretation of a pathological nature for such cases. To bring such cases into line with Christman's interpretation of a cell fusion, the explanation indicated above appears reasonable, viz. that sometimes the fusion of the two gametes begins through a narrow opening, so that the nucleus in passing through the pore becomes much elongated and constricted. Thus, in the light of the further facts brought out in this paper, the seemingly divergent accounts of Blackman and Christman become, in my opinion, easily reconciled, at least for the diffuse caeoma type of Rusts. But I wish to emphasize at this point Christman's word of caution as to the probable occurrence of nuclear migrations of an undoubted pathological nature in the cup-like aecidia, since I have found a number of cases of migrating nuclei between the multinucleated cells of *Puccinia Cirsii-lanceolata*. Much still remains to be explained with respect to the aecidium-cup; and in view of the probably regular occurrence of multinucleated cells in young stages of the cup, it seems certain that neither Blackman's ('06) 'nuclear migrations' nor Christman's ('05) cell fusions at the base of every spore-row in *Uromyces Caladii* will serve to explain all of the complications which appear to prevail in this more highly differentiated type of Rust.

While I believe, with Christman, that the two fusing cells in the Caeomas are approximately equal, I think that his conception as to the relative position of the two gametes in these forms, as well as their equality, should be somewhat modified and broadened. In all of Christman's figures showing the sexual fusions the two gametes are placed upright and parallel to each other. In my figures illustrating the process (Figs. 26–40) the two cells are seen to occupy various positions to each other. Sometimes the gametes are side by side and parallel; while, perhaps even more often, one appears to be placed below the other, and seems to come up from below at varying angles. Christman further represents each of the fusing gametes as possessing a sterile cell as its tip (see, e.g. his Fig. 4, '05), although the evidence, after a mutual examination of his preparations by the author and by myself, has not convinced me that he is right on this point. Wherever a sterile cell is shown in my figures (Figs. 26–28, 31, 33, 37, 38, 40), but one gamete is seen to possess such a cell; whereas the lower gamete in these preparations apparently lacks entirely this structure. These observations tend, therefore, to confirm in this one respect those of Blackman, in that he found one gamete only bearing a sterile cell as its tip. It will be remembered that the latter ingeniously suggests that this two-celled structure is a female reproductive organ, the upper sterile cell of which he regards as the now functionless trichogyne, while the lower cell retains its primitive character as a sort of egg, or 'female cell', which is fertilized by what Blackman terms a 'vegetative cell' from below. Blackman points out that the 'sterile cell' sometimes pushes up as a long slender growth between the epidermal cells, thus suggesting, in his opinion, its primitive function when it once pushed its way to the surface and there served as a trichogyne to bring the spermatium into relation with the female cell below.

While I agree to some extent with Blackman's observations of facts, I think that a much simpler interpretation should be applied to such structures than that proposed by him. I should agree with him that but one of the two gametes ordinarily bears a sterile cell, since I have never found convincing evidence that both the fusing cells were thus equipped, as maintained by Christman. In *Triphragmium* sometimes two such cells will be found in a row at the top of one of the gametes. But whether sterile cells are necessarily present, or whether, on the other hand, they may sometimes be altogether absent, as might be inferred from those drawings of cell fusions in which they are not shown (Figs. 32, 34–36, 39), I am not prepared to say. I believe, however, as indicated above, that in all the caeoma type of Rusts examined by me such a sterile cell is generally present at the tip of one of the gametes. Further study of the pseudoparenchyma in the aecidium-cup type will probably assist materially in the interpretation of these sterile cells, since the pseudo-parenchyma

cells seem to be cut off in such instances (see Fig. 11) more or less indefinitely from certain well-nourished hyphae which line the bottom of the cup. To all appearances this process is quite similar to the cutting off of sterile cells in the caeoma forms.

With Christman, I should regard such sterile cells as having but little phylogenetic significance, and as serving merely as 'buffer cells', to assist in rupturing the epidermis. Such sterile tips, at least in the case of the caeoma type of Rust, are clearly gametophytic cells which finally die from lack of food, or which fail of fertilization. But the presence of a sterile cell at the tip of *only one* of the fusing cells in the caeoma and not on the other has, in my opinion, an important physiological significance which has not heretofore been pointed out. This phenomenon indicates, to my mind, that those hyphae which bear such cells at their apices grow up from below some time *before* those hyphae which do not possess such a structure. The first gametophytic cells to group themselves more or less regularly upright and parallel under the epidermis of the host, apparently thus grow vegetatively for a time and cut off sterile tips; but so far as my observation goes, these early hyphae do not fuse among themselves. Other distinct hyphae appear to grow up later from below and to press in between the earlier growths, thus providing the fertilizing gametes. That the conjugating pairs of gametes are thus differentiated in time of development, is clearly indicated by the two highly significant facts noted above; viz., that one of the gametes bears a sterile cell while the other does not, and, secondly, that one gamete, in my experience, generally lies somewhat below the other. To these points might be added also the important fact that after the earlier hyphae are grouped under the epidermis a period of considerable duration undoubtedly elapses before the binucleated condition arises.

These facts, in my opinion, point to the interpretation just mentioned as a more simple and reasonable one than that proposed by Blackman. The latter's idea that the sterile cells are abortive trichogynes, while of great interest and theoretic importance, has therefore but little basis in fact.

It is quite evident that the nuclear divisions described above are to be classed as indirect divisions rather than amitotic, as Blackman regards them in the forms he has investigated. The latter author studied examples in which the chromatin in each vegetative nucleus was fused together so as to form a dumbbell-shaped mass and was otherwise poorly differentiated during division. Such a condition is apparently common among Rusts, since every one of the previous investigators who had studied the subject described similar appearances to those he figures. Holden and Harper probably rightly conclude that, in such vegetative mitoses, poor fixation must be responsible for the lack of differentiation. Blackman, on

the other hand, concludes that chromosome-formation in these cases 'is in complete abeyance; so that the division actually partakes of the nature of direct division' ('04, p. 356). In all the various types of Rust cells herein described—in those of the spermogonium and in the uninucleated cells of the gametophyte, as well as in the binucleated cells of the sporophyte—the mitoses are found to conform quite closely to the type figured by Hermann for the Salamander. All three of the species which I have carefully examined for the mitotic phenomena agree in this respect; and, although it is true that the nuclei of various species of Rusts vary greatly in size as well as in favourableness for study of the details of the process, it is highly probable that the phenomena as observed in *Triphragmium* will be found to be characteristic for the group.

As was described above for *Triphragmium*, the central spindle arises between the halves of the divided centrosome. Further, since the centrosome is located on the nuclear membrane, in a somewhat similar position to that occupied by the centre in *Phyllactinia* (Harper, '05), the central spindle comes to be formed in a corresponding position, along the nuclear membrane (Figs. 1–4). Soon the nuclear membrane breaks down and the central spindle thus comes to lie as a dense, strongly-developed structure at one side of the irregular mass of chromatin (Figs. 13–15, 24). It will be remembered that Blackman thinks that the spindles in the case of the promycelial divisions in *Gymnosporangium* are formed outside of the nucleus, free in the cytoplasm, between the two portions of a divided extra-nuclear centrosome. His figure illustrating the phenomenon resembles closely the nuclei of my Fig. 24. From the facts gained from a study of the earliest prophases in the spermogonium of *Triphragmium*, I am inclined to think that Blackman has missed the earliest stages of spindle-formation. It is quite probable, in my opinion, that *Gymnosporangium* will be found to agree with *Triphragmium* in having the centre located on the nuclear membrane, during the earlier stages, instead of outside the nucleus, in the cytoplasm. On the breaking down of the nuclear membrane, an appearance similar to Blackman's figure would then be presented. It is highly probable, further, that the description of the nuclear and cell division in the spermogonium of *Phragmidium violaceum* given by Blackman will be found to be based on poor preparations, since both processes in the case of the spermogonium of *Triphragmium* are perfectly normal and well differentiated. The division of the spermogonial nucleus in *Triphragmium* corresponds in all the essential features to the mitoses in other types of cells of this species; and further, the cell division by means of which the spermatium is cut off presents no such peculiarities as Blackman describes. The latter figures a sort of thickening ring on the hyphal wall below the constriction which he thinks may somehow be connected with the disjunction of the spermatium. This ring does not

appear at all in *Triphragmium* ; on the contrary, cell division takes place by means of the usual process of constriction.

Blackman's idea that we have in the Rusts three distinct kinds of nuclear division—the vegetative division, in the spermogonium and in the vegetative hyphae, which he regards as of the nature of amitosis; and the two promycelial divisions, which he thinks are reduced forms of indirect division—is apparently based on the study of unfavourable material. I have not yet compared the nuclear division in the promycelium of *Triphragmium* with the vegetative divisions of this form ; but since the latter are found to conform in the main features with the mitotic phenomena as described for other organisms, I think it more than likely that the promycelial and vegetative divisions in the Rusts will be found to differ from each other only in those characteristics which distinguish the heterotypic and homoeotypic divisions from the vegetative.

In *Triphragmium* the fibrous structures seen in the telophases, which represent the chromosomes, are seen to number invariably eight (Figs. 6 *a* and 10). Poirault and Raciborski's ('95) conception as to the single longitudinally-split chromosome of the Rust nuclei, as well as Sappin-Trouffy's ('96), and Maire's ('00) conclusion that the two chromatin masses so commonly observed represent two chromosomes, results without doubt from the study of poorly-differentiated mitotic figures. The well-differentiated nuclear figures obtained in the vegetative cells of *Triphragmium*, as well as the occasional favourable cases in some other forms, furnish convincing evidence of the correctness of Holden and Harper's view that the more or less solid dumbbell-shaped chromatin masses, characteristic of the vegetative nuclei of so many Rusts, result from poor fixation and poor differentiation. Blackman ('04) also regards the two chromatin masses in each nucleus as resulting from poor differentiation. He did not succeed, however, in making out distinct chromosomes ; nor did he see more than a 'simple spindle' in the achromatic structures.

Blackman offers without further comment the pregnant suggestion that the two chromatin masses first noted by Sappin-Trouffy 'probably represent the chromatin derived respectively from the two nuclei which fuse in the teleutospore' ('04, p. 346). The double centres seen in Figs. 6, 6 *a*, and 10, to each of which four chromosomes are seen to be attached, result, in my opinion, from this tendency for the chromatin contents of each daughter-nucleus to segregate into two more or less distinct masses. But to the views expressed in certain recent important investigations on related problems bearing on the cytological aspects of heredity in the higher plants (Allen, '05 ; Strasburger-Allen-Miyake-Overton, '05) Blackman's theory would present a striking contrast. The double character of the nuclei in the case of higher plants, resulting from the close proximity in the same nucleus of the chromatin derived from the two parents, clearly persists

throughout the sporophytic development. But here, in the Rusts, Blackman would have a doubling to result from a similar origin and to persist as well throughout the gametophyte. The nuclei of the vegetative gametophytic hyphae (Fig. 10), as well as those of the spermogonium (Figs. 6 and 6*a*), clearly show in some instances this phenomenon. The nuclear fusion in the teleutospore and the two promycelial divisions which presumably accomplish the reduction processes, have occurred but a short time before in the development of the fungus. While Blackman's conclusion might therefore seem to be the natural one under the circumstances, viz. that the double character of the nuclei results from the continued association of the same nuclear cavity of the chromatin derived from the two nuclei which have just fused in the teleutospore, the important bearing of such a theory and its lack of harmony with the recent theoretical work on the higher plants above mentioned, compels us to await more facts before accepting his conclusion. Another view is possible to explain the phenomenon. The double centre may be reasonably explained as resulting from a precocious division of the centre in each daughter-nucleus, in preparation for the next mitotic division—a phenomenon which has already been described as taking place in the nuclei of a number of organisms.

While not yet prepared, as indicated above, to discuss fully the important questions which arise as to the mode of origin of the multinucleated cells at the base of the aecidium-cup, nor as to the part they bear in the development of the aecidia, it may be advisable in this place to point out a few pertinent facts and theories. Massee ('88) claims to have seen such a multinucleated cell at the base of the aecidium-cup, which he thinks resulted from the fertilization of an oogonium by an antheridium. Richards ('95) has figured in a similar position one, or in large cups often more, large, sometimes multinucleated cells, which, according to him, give rise by budding to the basal cells at the bottom of the spore-rows. Richards thought that the multinucleated cells arose simply by the swelling up and growth of one or more special 'fertile hyphae' at the base of the cup. Blackman ('04 and '06) saw a number of cases in young aecidia in which the cells had three and four nuclei, but he regarded such isolated instances as abnormal.

That multinucleated cells are formed, probably as regular occurrences during the earlier stages in the development of the young aecidia, is indicated by their discovery, during the course of this investigation, in at least eight or ten species of Rusts. I am therefore convinced that they are perfectly normal occurrences, and that they result simply from the nuclear divisions going on much faster and thus getting ahead of cell division. Even in the caeoma form—*Triphragmium ulmariae*—instances, probably of a similar nature, have been occasionally noted in which five or six nuclei occur in one cell, although but two is the usual number.

Whether such multinucleated cells result from the stimulated growth

which follows the sexual cell fusions, or whether they are formed during the gametophytic stage, preceding the fusions, I am not prepared to say. The studies so far made on this point are only partly convincing; but, as mentioned above, the few facts at my command incline me to the belief that the multinucleated cells are sporophytic structures and that they result from the stimulated growth that follows the sexual cell fusions. Leaving aside for the present, however, the question of their origin, it is quite obvious that their seemingly normal and regular occurrence during the development of the young aecidia argues strongly against the acceptance of either Blackman's or Christman's ideas as to the mode of origin of the binucleated condition in the aecidium-cup Rust. At any rate, it is evident that further investigations of the minute structure and development of further types of the aecidium is needed before we can hope to bring this more complicated type into relation with the simpler caeomas.

Summary of Results and Conclusions.

1. The seemingly conflicting results obtained by Blackman and Christman, in their investigations of the sexual phenomena in the caeoma type of Rusts, are to some extent brought into harmony by certain new and supplementary facts recorded in the present paper. The two fusing gametes, as well as their nuclei, are regarded, however, as approximately equal, therein affording in greater part a confirmation of Christman's conclusions. While agreeing also with some of Blackman's observations of facts, certain of his conclusions with regard to these observations are not supported; e. g. that one of the fusing cells is generally smaller than the other, and that this smaller cell, which he thought contained a smaller, denser nucleus, is to be regarded as a 'vegetative cell', as distinct from the larger 'fertile cell'. Blackman's further theory that his so-called larger cell constitutes a 'female cell', and that its sterile tip is phylogenetically a 'trichogyne' also lacks support in the facts brought out in this investigation.

While the conjugation is therefore regarded as taking place between two gametes which are essentially similar in size, several observations in connexion with the sexual fusions, apparently noted only in part by Blackman and Christman, point to the conclusion that the two gametes differ somewhat in time of development. The observations on which this conclusion is based are as follows: (1) In the caeoma forms the first hyphae to push up under the epidermis mass themselves often more or less regularly upright and parallel and then proceed to cut off sterile cells at their tips. The sterile tips push up against the epidermal cells and soon degenerate. A more or less prolonged period of vegetation appears thus to intervene before the conjugations begin. (2) Generally only one of the two conjugating gametes bears such a sterile tip, while the other shows no such

differentiation. (3) The gamete which bears the degenerating tip-cell often appears to be placed somewhat above the other, thus suggesting that the earlier hyphae fuse, not among themselves, but with other hyphae which push up later from below.

This explanation appears simpler and more reasonable to the writer than the complicated theory proposed by Blackman. The sterile cell, according to the views advanced in the present paper, is not an abortive, functionless trichogyne, but merely a 'buffer cell'—a degenerate gametophytic cell, morphologically similar to the functional gametes. The simpler theory of course leaves the so-called spermatia as yet unexplained.

Conjugation is accomplished, as Christman maintains, through the absorption of a portion of the walls of the two gametes which are in contact. The process may begin, however, through a very small conjugation pore, so that as the one protoplast moves through the narrow opening to fuse with the adjoining gamete, the nucleus may thus sometimes be drawn out and constricted ; in this condition presenting the appearance which Blackman has termed 'nuclear migration'. Such an instance is regarded, however, merely as a case of conjugation of two cells in which the connecting pore is as yet small. Instances were observed in which a Blackman type of conjugation, as it may be termed, through a narrow pore, occurred side by side with a Christman type of fusion, through a broad pore. The essential feature of the conjugation process in the caeoma forms is therefore regarded as the equal participation of two morphologically equivalent cells to form a binucleated double cell—the so-called 'fusion cell'.

2. In the present investigation, the vegetative nuclear divisions have been studied in the spermogonia and in other gametophytic hyphae, as well as in the binucleated sporophytic hyphae. The process is essentially the same in all these types of cells, being a mitotic phenomenon, and not partaking of the nature of amitosis, as Blackman claims for the nuclei of *Phragmidium*. Each nucleus, during the conjugate divisions, acts apparently in entire independence of its associated nucleus. During the earlier stages of the association of the two nuclei in the one cell, just following the sexual fusion, the mitotic figures may sometimes be variously oriented in the cell, bearing no obvious relation to each other. Later, however, the two conjugate spindles generally arrange themselves more or less regularly parallel to each other in the long axis of the cell.

Each nucleus divides by the aid of a centrosome, which is located on the nuclear membrane, and which in some forms persists in the resting stages as a distinct point of polarization of the nuclear contents. During the prophases, the centre divides and the two daughter-centres move apart on the nuclear membrane. Between the diverging centres a filamentous structure appears—the central spindle of Hermann. Soon the nuclear membrane breaks down and the central spindle comes to lie as a dense,

thready structure at one side of the chromatic mass. Mantle fibres now extend out from the diverging polar centrosomes to the chromatin and accomplish the division into the two daughter-halves. During the later phases, a few polar radiations generally appear.

While the chromosomes in the case of *Triphragmium* are not sufficiently differentiated in the equatorial-plate stages to be definitely counted, during the anaphases, however, the chromatic radiations which converge to the poles become quite distinct and are then seen to number eight. These chromatic strands are regarded as corresponding to chromosomes. Further, they are often seen to be segregated into two distinct groups of four each, and each group is seen to be attached to a distinct centrosome. This double character of the daughter-nucleus is interpreted as expressing in somewhat clearer terms the doubling noted by several earlier writers. Rather, however, than denoting the longitudinal splitting of a single centrosome, as held by Poirault and Raciborski; or the existence side by side in the one nucleus of two distinct chromosomes (Sappin-Trouffy and Maire); or the presence in the same reduced nucleus of the chromatin derived from the two parents (Blackman), it is suggested that the double character may result from a precocious division of the centre, in preparation for the next mitosis.

3. The apparently normal and regular occurrence at the base of certain young aecidia of one to many multinucleated cells, points to the necessity of a broader conception as to the mode of development of the aecidium-cup than that held by either Blackman or Christman. While the part which these multinucleated cells take in the development of the aecidium is as yet somewhat obscure, the evidence appears to point to the conclusion that they are sporophytic structures and that they result from the stimulated growth which follows the sexual cell fusions. Should this prove true, it is obvious that the 'fusion cell' does not at once function as a 'basal cell', at the bottom of each spore-row, as maintained by Christman for this type of Rust. Further, the occurrence of occasional instances suggesting 'nuclear migrations', undoubtedly of a pathological nature, between the multinucleated cells of *Puccinia Cirsii-lanceolati*, throw doubt on the idea as to the normal origin of the binucleated condition in the aecidium-cup by this means.[1]

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[1] Since the above went to press, two papers on the subject (The Relation of 'Conjugation' and 'Nuclear Migration' in the Rusts; and The Relationships of the Aecidium-cup Type of Rust) were read by the writer before the Chicago meeting (1908) of the American Association for the Advancement of Science, abstracts of which are to be found in Science XXVII: 213–15, Feb. 7, 1908.

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EXPLANATION OF PLATE XXII.

Illustrating Professor Olive's paper on the Rusts.

All the figures were drawn with the aid of the camera lucida, and, except the few specially noted below, with Zeiss Apo. Obj. 2 mm. N. A. 1 : 30, combined with Compens. Oc. 12, thus securing a magnification of 1500 diameters. Various Compensating Oculars, combined with the Apo. Obj. 2 mm. N. A. 1 : 40, were used for those figures in which the magnification is specially designated.

(Except where otherwise stated, all figures are drawn from *Triphragmium ulmariae*.)

Fig. 1. Nucleus from a spermogonium, showing on the nuclear membrane and enclosed in an archoplasm-like substance, two deeply-stained centrosomes, on each of which a certain amount of chromatin is centred.

Fig. 2. Dividing centres, connected by a slender strand—the central spindle. The nuclear

membrane appears to be still intact, and the centres and the central spindle are evidently on its upper surface.

Fig. 3. The central spindle now reaches to the poles of the figure. The lower pole is obviously double. The chromatin and nuclear sap lie mainly in the swollen lower half of the mitotic figure, while the upper part, in the forming spermatium, appears to be made up almost entirely of a fibrous material. × 2250.

Fig. 4. Early anaphase. The densely fibrous central spindle is at one side of the axis of the figure. The chromatin is being drawn to the poles somewhat irregularly. The lower pole shows some astral radiations.

Fig. 5. Poorly differentiated late anaphase, from the spermogonium of *Uromyces Lilii*, showing central spindle, mantle fibres, budding spermatium, &c. × 2250.

Fig. 6. Late anaphase, from *Triphragmium*, showing at both poles the double centres and mantle fibres. The two fibres which extend between the daughter-nuclei are probably a remnant of the central spindle.

Fig. 6 *a*. A double centre, more highly magnified, showing four chromatic strands attached to each half, thus making eight chromosomes to the daughter-nucleus. × 2250.

Fig. 7. A spermatium, showing its comparatively large nucleus, with a nucleole and centrosome. × 2250.

Fig. 8. A highly magnified resting (?) nucleus from a multinucleated sporophytic cell of *Puccinia Cirsii-lanceolati*, showing the polarized condition. × 3375.

Fig. 9. Poorly defined equatorial-plate stage of division, from the spermogonium of *Uromyces Scirpi*. × 2250.

Fig. 10. Telophase of the mitosis in the gametophytic hypha which cuts off the so-called 'sterile cell'. The centre in the upper nucleus is distinctly double, and four chromosomes are attached to each half. The lower nucleus is seen in obliquely polar view, so that here also about eight chromosomes may be counted, radiating from the centrosome.

Fig. 10 *a*. Anaphase of a similar nuclear division.

Fig. 11. Telophase of a mitosis in a gametophytic hypha at the base of the aecidium-cup of *Uromyces Scirpi*. The uninucleated cell which will be cut off at the tip will form a 'pseudo-parenchyma' cell.

Fig. 12. Prophases of a conjugate division in a fusion cell. The nucleole is apparently about to be cast out from the left nucleus. Each nucleus shows (probably on its surface) a dumbbell-shaped, dividing centrosome. The strand connecting the two centres doubtless represents an early stage of the central spindle.

Fig. 13. Prophases of conjugate division showing the two nucleoles which have been cast out into the cytoplasm; the two central spindles, the poles of which are each terminated by a disk-shaped centrosome; and the mass of chromatin attached to each spindle.

Fig. 14. A later stage in which the two spindles lie almost at right angles to each other. The nuclear figure at the left is well oriented to show the dense, curving, central spindle, terminated by broad centrosomes, and a few mantle fibres, which reach out from the central spindle, apparently to pull the chromatin finally to the poles.

Fig. 15. The double base suggests that two cells have here fused to form the budding 'basal cell', although no partially absorbed walls remain to confirm the suspicion. The stage in conjugate division here represented is somewhat similar to that shown in Fig. 14. The central spindles in this instance are, however, unlike the latter case, placed parallel to each other.

Fig. 16. Early anaphase stage of conjugate division in a young peridial cell from the aecidium-cup of *Uromyces Scirpi*. The two nucleoles lie near the equator of the central spindles, a position very commonly assumed in conjugate division. The chromatin in this instance shows the usual poor differentiation which seems to be characteristic for vegetative divisions. × 2250.

Fig. 17. Late anaphase, showing broad-poled spindles, mantle fibres, asters, and vacuolated nucleoles.

Fig. 18. Still later stage of conjugate division. The upper right-hand nucleus has a very conspicuous double centrosome.

Fig. 19. Telophase of conjugate division. About eight chromosomes may be counted, radiating from the centre in each nucleus. The companion of the right nucleus lies in a lower plane.

Fig. 20. Late stage of conjugate division in a basal cell from the aecidium on *Cicuta*. Note the centres, the remnant of the central spindle, &c.

Fig. 21. Telophase of a double conjugate division in the *Cicuta* aecidium. The paired arrangement of the four spindles i apparently only partially maintained.

Fig. 22. Four reconstructing daughter-nuclei in a basal cell of *Puccinia Cirsii-lanceolati*. In three of the nuclei the centres still have an aggregation of chromatin about them.

Fig. 23. Simultaneous multiple division in a multinucleated cell at the base of the aecidium of *Puccinia Cirsii-lanceolati*. Besides the nine poorly differentiated nuclei shown in the figure, three are to be found, belonging to this same cell, in the next section. No paired, or conjugate, relation of the nuclei is here apparent.

Fig. 24. Simultaneous division of six nuclei (three more lie in a lower plane and are consequently not shown in the drawing) in a multinucleated cell at the base of the aecidium-cup of *Uromyces Scirpi*. The four upper mitotic figures show clearly the irregular mass of chromatin attached to one side of each central spindle. Here again no paired arrangement is apparent.

Fig. 25. A multinucleated cell, from the base of the aecidium on the *Thistle*. × 960.

(Figs. 26–40, showing sexual cell fusions, have been so oriented that the upper part of each figure points toward the top of the plate as toward the epidermis of the host.)

Fig. 26. Two pairs of fusing cells. Above the upper gamete of the right pair is a 'sterile cell'.

Fig. 27. Fusion of two cells, and the apparent migration of one nucleus over into the right half of the 'fusion cell'. A large degenerating sterile cell has been cut off from the tip of the right gamete.

Fig. 28. Cell fusion, in which the lower of the two gametes apparently arises from a hypha which comes up obliquely toward the eye. A budding growth has pushed out above, to one side of the sterile cell, before the passage of the lower nucleus into the upper part of the fusion cell.

Fig. 29. Basal fusion cell, showing, below, the remnant of the absorbed partition wall which formerly separated the two gametes. One spore 'mother-cell' has been budded off and a second bud has started off to the left. A detached, crushed sterile cell lies between the two buds; whether this originally tipped the upper of the two gametes is uncertain.

Fig. 30. The partially-absorbed partition wall which once separated the two gametes is here seen to be peculiarly bent and contorted.

Fig. 31. One gamete appears to lie obliquely below the upper gamete, and the absorption of the wall separating them has apparently just begun. The upper gamete bears an unusually large sterile cell, the contents of which, excepting the nucleole, appear to have almost entirely degenerated.

Fig. 32. Fusion cell in which the conjugation pore is clearly shown. The two conjugate nuclei have entered upon the prophases of division, as evidenced by the fact that one nucleus has cast out its nucleole and left it behind in the lower gamete.

Fig. 33. An instance of cell fusion in which the nucleus of the lower gamete has just begun to pass through a very small pore into the upper gamete.

Fig. 34. A similar instance, in *Gymnoconia interstitialis*, in which more than half of the lower nucleus has passed through a small conjugation pore. The conjugating hyphae are evidently obliquely placed with respect to each other.

Fig. 35. Cell fusion in *Gymnoconia* in which the two conjugating cells lie side by side and parallel to each other. One has grown up a little higher than the other. A dense, finely granular aggregation of protoplasm lies in the fusion cell where the conjugation pore was formed. The centrosome of each nucleus is dumbbell-shaped and is in process of division.

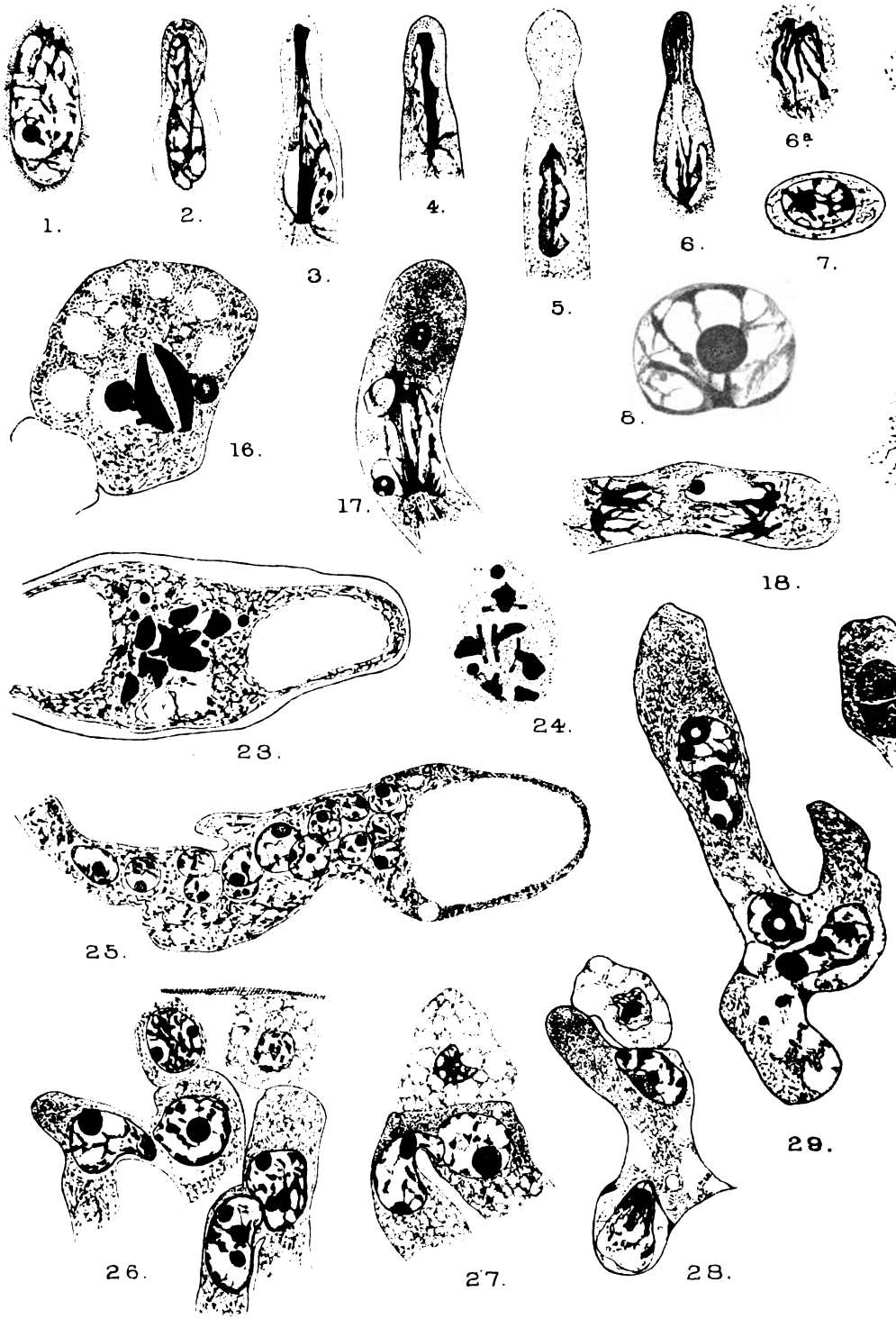
Fig. 36. A similar fusion cell in *Gymnoconia*, from the same preparation as the one shown in Fig. 34.

Fig. 37. The beginning of the formation of the conjugation pore in *Phragmidium potentillae-canadensis*. A deeply staining body, somewhat similar to a nucleole, lies in the pore.

Fig. 38. Fusion cell in *Phragmidium potentillae-canadensis*, which has begun to grow out to one side of the sterile cell.

Fig. 39. Fusing tips of two hyphae of the *micro*-form, *Puccinia transformans*. Excepting for their nucleoles, the two nuclei are poorly differentiated.

Fig. 40. Cell fusion in *Puccinia transformans*, in which an end cell of one hypha has fused with a penultimate cell of another hypha.



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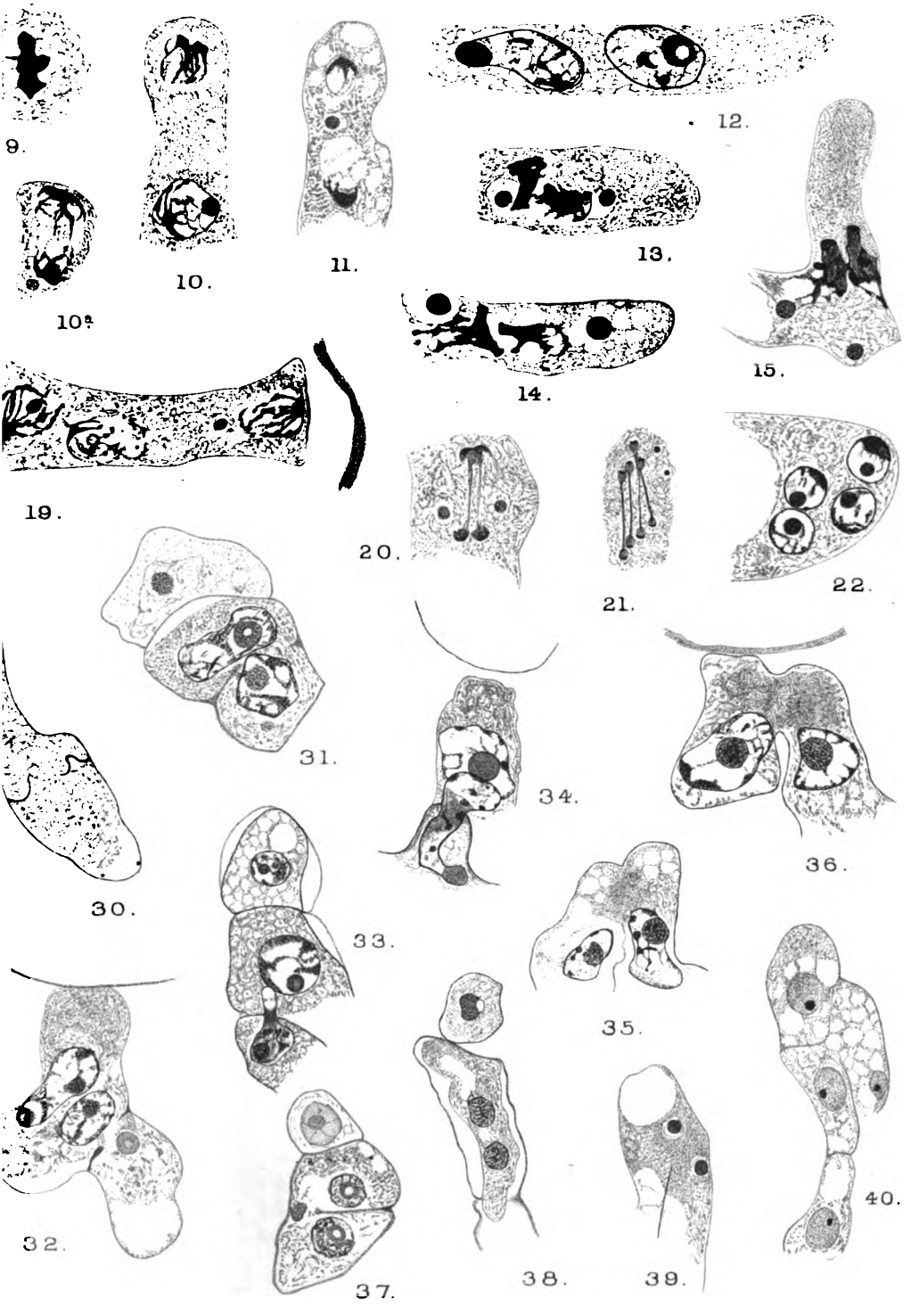

Huth lith et imp

A Contribution to the Physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs.[1]

BY

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With Plate XXIII.

THE Saprolegniaceae have been a favourite subject for investigation by some of the most prominent botanists of the last century. Unger ('43), Schleiden ('42), and Naegeli ('47) were among the first contributors towards a knowledge of these fungi, while Pringsheim ('57, '60, and '74) and De Bary ('81) gave them the first extended morphological and systematic study. In 1872 Cornu began a monograph which he never finished. De Bary gave them special attention up to the last years of his life, and his accurate observations and experiments were reported posthumously in 1888 by Solms-Laubach. In this country Humphrey ('93) extended our knowledge of American forms and summed up the taxonomic facts in a monograph. In more recent years investigations have taken new directions: Trow ('95, '99 and '04), Hartog ('95 and '99), and Davis ('04) have attacked the problems of fertilization and of nuclear phenomena by means of modern cytological technique, while Klebs ('99 and '00) has obtained important experimental results towards a theory of reproduction.

It is upon the foundation laid by Klebs that the present investigation has attempted to build; and the following paragraph may be considered as presenting the lines along which advance seemed possible:—

(*a*) The method which Klebs applied to the study of a single species has been used for a comparative study of a number of species of the same genus. (*b*) The special problem of sex determination or at least of antheridial development has been approached with a species whose reproductive organs are more suitable for such study than those of *Saprolegnia mixta*. (*c*) An effort has been made to learn something of the taxonomic values of those characters which have been generally used in the genus

[1] Contribution ninety-three from the Botanical Laboratory of the University of Michigan.

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

Saprolegnia for purposes of classification. (*d*) The details of technique are not especially emphasized by Klebs, and this made it difficult to repeat his experiments with ease and rapidity. It was therefore found to be necessary to work out in detail the technique used, and it seems advisable to report the same, and so make pure cultures of the fungus more available for class use or for purposes of research.

Most of the experiments were done during the winter months of the last five years in the intervals of other work. I am indebted to Professor R. A. Harper, of the University of Wisconsin, who first suggested to me the line of work which has culminated in this paper. My thanks are also due to Professor G. F. Atkinson, of Cornell University, and to Professor F. C. Newcombe, of this laboratory, for their continued interest and assistance.

TECHNIQUE.

The methods of culture used by De Bary, Humphrey, and others usually afford material for study which is fairly reliable, as it can be obtained approximately free from bacteria and other fungi. No cultures had, however, been made from a single isolated zoospore or gemma up to the time of Klebs's paper, and no method has as yet been outlined for cultures with single zoospores. In only one instance have I come across a reference to a possible method of zoospore culture. Trow ('95) isolated a single zoospore of *Aphanomyces* by means of cover-glasses on which white of egg had coagulated in thin coatings, and which had been floated on water containing *Saprolegnia*, &c. A single culture resulted. On the other hand, cultures have been obtained from masses of zoospores presumably originating from a single sporange (Maurizio, '96); but in all such cases the possibility of contamination with zoospores from other species is great.

Maurizio ('94) isolated the various species by snipping off the ends under a lens before the formation of sporanges. From a bundle of hyphae cut off in this way and floated in water a single hypha was removed by drawing up into a pipette and transferring to a fly's leg on a slide. The water on the slide was changed every day by the use of filter paper. If this did not yield a single species the process was repeated. His substratum during this study were meal-worms, flies, and cress-seedlings. In 1896 he made a considerable variety of cultures on peptone, cane sugar, milk sugar, egg albumen, Liebig's extract, beef broth, and glycerine, all of which flourished. Boric and salicylic acid were used to keep off the bacteria, and this indicates that his work was not always done under sterile conditions. Trow ('95) used the same method of isolating hyphae, usually selecting such as had oogonia on them.

Klebs ('99) isolated a single gemma of *Saprolegnia mixta* and under entirely sterile conditions succeeded in studying the development of a single

species in great detail. He found it grew very luxuriantly on the various substances which Maurizio had used, and evidently had no trouble in keeping it sterile, once he had isolated the gemma.

Davis ('04) does not give any details as to how he isolated his material. He does not seem to insist on its having been entirely sterile at all times.

My own method was gradually developed from the hints thrown out by preceding investigators, and although far from meeting all the requirements of the whole family, it has made it possible to isolate the species of the genus *Saprolegnia* from both bacteria and other species and genera of the water moulds with great ease and certainty.

The fungi were obtained, as is usual, from water brought in from as many habitats as possible—river, ponds, lakes, ditches, brooks, and springs —and contained algae or other aquatic plants or merely decaying vegetable matter. The separate collections were placed in jars in the laboratory which were then filled up with distilled water. The tap water is well known to be deleterious to many algae, and they do much better in their native water or in distilled water. The jars were all labelled, and into each was thrown a fly or a small piece of dry beef; the former is better, as bacteria attack the beef too quickly. After being exposed twenty-four hours this bait was usually found to be inoculated, especially if the jars, which were kept in a cool window, had been warmed up somewhat by the sunshine. It is well to expose the bait as short a time as possible. The fly or meat was then transferred to sterile glass capsules containing distilled water, and in a few days was covered by the characteristic zone of the fungus. To remove the bacteria, a piece of the mycelium was cut off with scissors and transferred to a Petrie dish containing beef-gelatine.

As satisfactory results are only obtained from the right sort of beef-gelatine, the method of preparation may be given at this place. About half a kilogram of fresh lean beef is chopped fine and placed in a glass jar and one liter of distilled water poured over it. After standing twenty-four to thirty-six hours in a cool place the water is siphoned off and to every 500 cc. is added 52 to 55 grams of sheet-gelatine. The mixture is then heated on the water bath and stirred till all the gelatine is dissolved. After allowing it to cool to between 45° and 50° C., white of egg is added and the mixture thoroughly shaken to a froth, when it is placed in a steam sterilizer and boiled thirty minutes. It is then filtered through several layers of filter paper into Erlenmeyer flasks as stock material. The gelatine should be perfectly clear and become hard when set out into the ordinary room temperature. Sterilization is repeated on the two following days. From the stock material a thin layer is poured into Petrie dishes from time to time, and these are used to isolate the fungus.

The Petrie dish containing the impure mycelium is now placed on

a cold window-sill or in a refrigerator at a temperature between 5° and 10° C. or lower. The mycelium immediately begins to grow vigorously, while the bacteria multiply slowly, so that in from twelve to eighteen hours the margin of the mycelial mass outstrips the bacteria by as much as 4–6 mm. From the outer zone a piece of gelatine containing the ends of hyphae is cut out with a slender scalpel, and transferred to a clean dish, where it will again grow rapidly. Usually this is sufficient to separate the bacteria; if there are still some present the process is repeated. The new growth may appear to contain bacteria because of the action of proteolytic enzymes which soften the gelatine around the new culture; this appearance is still more exaggerated by the use of some commercial extracts of beef whose transformations produce a turbid liquid. It may be added that no lactic acid or other antiseptic was used, although the mycelium grows in acidified solutions; it seemed desirable that no stimuli of such a kind should be present before the regular experiments were begun.

If now there is only one species present, it is easy to obtain a culture from a single zoospore, and even if several species are present one of the number can be easily separated. A piece from the margin of the Petrie dish culture which is free from bacteria is transferred into sterile water in a small glass capsule, where, after twenty-four hours, it will be found to have produced sporanges and zoospores abundantly. The spores are more active and escape more readily from the sporange if the capsule is placed in a warm place a short time before using. The water is found to be swarming with spores and can be taken up in a sterile pipette and a drop of it mixed with 20 cc. of sterile water; from this diluted fluid a pipetteful is again taken and squirted over the surface of a clean gelatine plate in as fine droplets as possible. After another twenty-four hours or less one can find germinated spores lying far from any other spores, and these are then cut out with a very narrow and pointed scalpel and transferred to a gelatine dish as the final step in separation. We now have a culture from a single zoospore, and all further experiments can be made on mycelium obtained from it.

As all the species which were studied produced so-called gemmae, these may be used in the manner of Klebs and Trow as the starting-point of cultures. Their method, however, requires more skill and is to be preferred only where it seems difficult to get zoospores. It can also be used where it is desired to get a definite species from a mixture of several species. As a last resort, the method outlined by Maurizio is available.

The results of the present paper were obtained from cultures which were started from single spores. After the isolation was accomplished, the work was done with scrupulous care as regards sterilizing the instruments, glassware, and solutions used. It is very easy to transfer accidentally spores or bits of mycelium from one set of cultures to another, and hence mix up

the different species. Whenever cultures became contaminated in any way they were immediately discarded. Contamination from other species of *Saprolegnia*, however, never occurred, since the instruments, &c., were always heated between using. Only in this way can permanent results be obtained.

Experimental.

All the cultures started were labelled by a letter. The following letters represent pure cultures from a single spore: C, F, H, I, K, L, M. After the development of fruiting bodies an attempt was made to determine the species; it resulted as follows:—

C. *Saprolegnia hypogyna*, Prings.
H. *Saprolegnia mixta*, De Bary . . . a form!
F. ditto ditto
I, L. *Saprolegnia*, sp.
K. *Pithyopsis*, sp. ?
M. *Aphanomyces laevis.*

C. *Saprolegnia hypogyna* was collected, October 25, on Pontederia at Cedar Lake, west of Chelsea, Mich. The culture was made from this material January 9.

In this species, which has not hitherto been reported for America, there is a short cell cut off by a cross-wall immediately below the oogonia. Maurizio ('94) studied this species, and also what he designated as five varieties, from a taxonomic point of view. After he had isolated a pure culture from the original, he found it so variable that he was led to begin the 'isoliren' anew, and obtained three forms closely related to *S. hypogyna*. This illustrates the shortcomings of his method. Later, he found other varieties from other sources. Only Pringsheim and De Bary had described the species before him, Pringsheim with a few figures as a variety of *S. ferax*, De Bary without figures but as a distinct species.

Cultures were immediately made to study its behaviour. A transfer was first made to a capsule of pea-broth, as described by Klebs, by sterilizing three or four peas in 50 cc. of water. After three days the mass of resultant mycelium was taken out, washed in sterile water, and divided into equal parts, which were then transferred to the various solutions or substrata.

For the sake of comparison with the work of other investigators, flies, wasps, and dry beef were also used in some cultures. On flies and wasps, *Saprolegnia hypogyna* formed sporanges and zoospores in great abundance after three or four days, of the characteristic *Saprolegnia* form and the method of emptying. In six to ten days the culture was full of oogonia. After a trial series of various substances, solutions of those substances were used which seemed to effect the production of sexual organs. The best substances, as found also by Klebs, were haemoglobin and leucin.

Solutions were made by mixing a ·05 per cent. haemoglobin solution and a ·2 per cent. solution of the inorganic salt desired, using half of each solution; this gave a solution containing in each case ·025 per cent. haemoglobin and ·1 per cent. of the salt. The following salts were tried: K_3PO_4, xKH_2PO_4, xNa_2HPO_4, $Ca(NO_3)_2$, xNH_4NO_3, KNO_3, $x(NH_4)PO_4$ $(Ca)_3(PO_4)_2$, $xNaH_2PO_4$, K_2SO_4, $xZnCl_2$. Also peptone in ·02 per cent. solution. Those marked with an x showed few if any oogonia. The results are based on the first eight days' growth:—

(*a*) Solutions with ·05 per cent. haemoglobin and no salts were tried first. Here the oogonia were abundant, with many oospores in each oogonium; the hypogynous cells were present but with hardly any side-branch.

(*b*) Haemoglobin and peptone. Oogonia very abundant with 2–10 oospores in each oogonium; the hypogynous cell beneath each oogonium had no side-branches, but there were projections into the oogonium.

(*c*) Haemoglobin and potassium phosphate. Oogonia abundant, but the oospores did not always mature; in such cases there was always a great abundance of antheridial filaments, which arose either from the hypogynous cell or from the neighbouring mycelium; hypogynous cell was either absent or present.

(*d*) Haemoglobin and calcium nitrate. Oogonia medium abundant; hypogynous cell was present; side-branches short or wanting, not developing into antheridia.

(*e*) Haemoglobin and potassium nitrate. Oogonia abundant with 1–10 oospores; hypogynous cell either present or absent; antheridial branches present, often luxuriant around the disintegrating oogonia.

(*f*) Haemoglobin and calcium phosphate. Oogonia abundant, with one to many well-formed oospores; hypogynous cell always present, sometimes several; no antheridial branches.

Another set of cultures contained ·05 per cent. haemoglobin, and the same series of salts as before, including ammonium tartrate and rubidium hydroxide. The results noted were obtained during the first eleven days of development:—

(*a*) Solutions with ·1 per cent. haemoglobin were used, containing no salts. Oogonia very abundant with 3–15 oospores in each oogonium; always with a well-formed hypogynal cell which is only rarely branched, and then the side-branch is short. A few cases of additional hypogynous cells were seen.

(*b*) Haemoglobin and ·1 per cent. peptone. Oogonia abundant, with 3–10 well-formed oospores; hypogynous cell always present, sometimes two cells; no antheridial branches.

(*c*) Haemoglobin and ·05 per cent. potassium phosphate. Oogonia abundant, with three somewhat disintegrated oospheres, but with antheridial filaments in great profusion around the oogonia.

Also, haemoglobin and ·1 per cent. potassium phosphate and ·1 per cent. sodium chloride; result as in the preceding, except more marked; about 75 per cent. of the oogonia have profuse antheridial filaments.

(*d*) Haemoglobin and ·1 per cent. calcium nitrate. Oogonia very abundant, with well-developed oospores; hypogynous cell always present, rarely a second cell; about 1 per cent. with short antheridial branches.

(*e*) Haemoglobin and ·1 per cent. potassium nitrate. Oogonia abundant, oospheres often poorly developed where the antheridial filaments are profuse; antheridial side-branch at times normally developed, with a well-formed antheridium.

(*f*) Haemoglobin and ·05 per cent. calcium phosphate. Oogonia abundant, but rather small, with only 1–5 oospores; hypogynous cell always present, rarely a second one; only 1 per cent. have short antheridial branches, with, however, no antheridia.

(*g*) Haemoglobin and ·1 per cent. potassium sulphate. Oogonia moderately abundant with well-formed oospores; hypogynous cell always present, rarely a second; only 1 per cent. with short antheridial branches.

The rubidium allowed only vegetative growth, which was moderately luxuriant. In the ammonium tartrate solution the oogonia were developed poorly.

For a special study of the effects of calcium and potassium sulphate, the following sets of cultures were made:—

(*c*) ·05 per cent. haemoglobin, ·1 per cent. potassium phosphate, and ·1 per cent. potassium sulphate. Oogonia fairly abundant, always with an hypogynous cell, and always with an antheridial side-branch which bears antheridia; often these antheridia are diclinous and surround the oogonia profusely.

(*d*) ·05 per cent. haemoglobin and ·1 per cent. calcium nitrate. Oogonia abundant with well-formed numerous oospores up to twenty in number; hypogynous cell either present or absent; antheridial side-branches few, short, and undeveloped.

(*f*) ·05 per cent. haemoglobin, ·05 per cent. monohydrogen calcium phosphate. Oogonia moderately numerous, from 1–15 oospores, oftener only a few; hypogynous cell with or without a side-branch, which, however, is never fully developed.

(*h*) ·05 per cent. haemoglobin, ·1 per cent. disodium hydrogen phosphate, and ·1 per cent. potassium sulphate. Oogonia medium numerous, with hypogynous cells; antheridial side-branches are present, and diclinous filaments with antheridia surround the oogonia as in (*c*).

H. *Saprolegnia mixta*, De Bary. This is apparently a form of the plant described by De Bary, differing in fly cultures mainly in having antheridia on 75 per cent. or more of its oogonia. A full description of the plant as it appeared in fly culture is herewith given.

Hyphae rather slender; zoosporangia nearly cylindrical. Oogonia with rather thick walls, terminal, intercalary or lateral, flask-shaped, rarely spherical, the lateral on short oogonial branches; pits medium to large, rather numerous but not easily seen; antheridial branches usually androgynous when the oogonium is lateral, diclinous when the oogonia are terminal or intercalary, long and slender when diclinous, short and slender and not coiled when androgynous; antheridia on 75 per cent. to 90 per cent. of the oogonia, long, subcylindrical, not very profuse on a single oogonium, often only one present. Oospores up to 15 and 20 in an oogonium, average diameter 24 microns, with rather thick walls.

The culture was obtained from a jar of Cladophora collected November 9, 1905, in the Huron River at Ann Arbour. The final culture from a single zoospore was made January 1, 1906.

This form or one of its near relatives was studied by Klebs, and the results published in the paper which instigated this one. My own results on this form corroborate most of his essential observations, although the most attention was paid to the production of antheridia with inorganic salts.

Cultures were made with solutions containing ·05 per cent. leucin, and ·1 per cent. inorganic salts. The observations include the first nine days of growth:—

(*a*) A 1 per cent. solution of leucin was first tried without any salts. Oogonia were abundant, and about 75 per cent. of them were provided with antheridia.

(*c*) Leucin and potassium phosphate. Oogonial initials were formed, but did not develop into maturity; gemmae formed in considerable numbers.

(*d*) Leucin and calcium nitrate. Oogonia fairly abundant; about 80 per cent. to 90 per cent. of them had antheridia; oospores numerous up to 15 and 18.

(*e*) Leucin and potassium nitrate. Oogonia abundant, antheridia on 70 per cent. of the oogonia; oospores numerous up to 25.

(*f*) Leucin and calcium phosphate. Oogonia abundant; antheridia on 90 per cent. or more, the oogonia densely covered by the antheridia in many cases; oospores not very well developed.

(*g*) Leucin and potassium sulphate. Oogonial initials present; antheridia not developed.

(*h*) Leucin and sodium chloride. Oogonia fairly abundant, with antheridia on 80 per cent. of them; oospores as many as 25 in each oogonium.

(*i*) Leucin and magnesium sulphate. Antheridia on 95 per cent. or more of the oogonia; oospores 10–20 in each oogonium.

Cultures with Na_2HPO_4, $(NH_4)_3PO_4$, NaH_2PO_4, and NH_4NO_3 had no oogonia; in the first three, gemmae formed in abundance. With $ZnCl_2$ in ·1 per cent. solution death resulted.

No haemoglobin solutions were carried through satisfactorily, so that the results on these are omitted.

F. *Saprolegnia mixta*, De Bary (form). This can also be considered as a form of De Bary's plant, differing from it on fly cultures by the large but comparatively few oogonia with many oospores, and by not more than 1–2 per cent. of its oogonia having antheridia, as well as in the somewhat different appearance of the vegetative zone.

The original culture came from a jar of Elodea obtained in a pool near the Huron River, Ann Arbour, November 9, 1905. The culture from a single zoospore was made January 1. The cultures were made in the following solutions, containing ·05 per cent. leucin and ·1 per cent. of the inorganic salts. Examinations were made during the first ten days:—

(*a*) Leucin ·1 per cent. solution, without salts. Only a few oogonia were found, and these had no antheridia.

(*c*) Leucin and potassium phosphate. Oogonial initials were present, but no oospores.

(*d*) Leucin and calcium nitrate. Several oogonia only were seen, with no antheridia.

(*e*) Leucin and potassium nitrate. Oogonia scattered, with many oospores each, a few with antheridial filaments.

(*f*) Leucin and calcium phosphate. No oogonia formed; gemmae finally appeared.

(*g*) Leucin and potassium sulphate. Oogonia scattered, large; about 25 per cent. with antheridial filaments both androgynous and diclinous.

(*h*) Leucin and sodium chloride. Several oogonia and a few antheridial filaments.

(*i*) Leucin and magnesium sulphate. A few large oogonia with many spores, some with antheridia.

Cultures with NaH_2PO_4, Na_2HPO_4, and $(NH_4)_3PO_4$ formed gemmae only; with NH_4NO_3 and $Rb(OH)_2$ they remained vegetative altogether.

A few cultures were also made with haemoglobin, using a ·1 per cent. solution, the salts as before:—

(*a*) Haemoglobin and peptone. Oogonia scattered, no antheridia.

(*d*) Haemoglobin and calcium nitrate. Oogonia abundant, no antheridia.

(*e*) Haemoglobin and potassium nitrate. Oogonia abundant; about 25 per cent. with antheridia, both androgynous and diclinous, sometimes from the oogonial stalk directly under the oogonium.

L. *Saprolegnia*, sp. As no oogonia were obtained in the cultures of this species, it was impossible to determine it. Under all the conditions which influenced the production of sexual organs in the species so far mentioned, this one remained either vegetative or produced abundant gemmae. In very few instances were sporanges or zoospores seen, the

former being usually transformed gemmae. Both leucin and haemoglobin series of cultures were tried. The sporanges were of the *Saprolegnia* type and emptied in the same manner.

The plant was obtained from mosses collected in a ditch near a peat bog at Chelsea, Mich., November 11. Since the mycelium did not respond readily when transferred to pure water in the formation of zoospores, the pure culture was made by transferring a single gemma as Klebs had done. This germinated by sending out from its anterior end numerous branches, while the hyphae at the end where it had been attached shrivelled up.

Gemmae were formed in greater or less numbers in nearly all the solutions of the series of haemoglobin. The solutions tried contained ·025 per cent. haemoglobin with ·1 per cent. peptone, laevulose, glucose, K_3PO_4, KNO_3, $CaHPO_4$, $Ca(NO_3)_2$, K_2SO_4, NaCl, $Cs(OH)_2$ respectively. Various combinations of these were also tried, but without success. With $Cs(OH)_2$ no gemmae were formed, while the vegetative growth was good.

Leucin was used with $Ca_3(PO_4)_2$, $Ca(NO_3)_2$, KNO_3, K_3PO_4, and $MgSO_4$. In all these leucin solutions, only gemmae were formed, often in great abundance. In shape the oogonia were often globular, as if they were oogonial initials, especially in the haemoglobin-laevulose and potassium phosphate and potassium sulphate solutions. The clusters of gemmae are short and much branched in many cases.

I. *Saprolegnia*, sp. This species reacted similarly to L, except that in one culture oogonia were seen. This occurred on a pure fly culture, but they remained immature and disintegrated, and no determination was made as to the species. The oogonia which were noticed were densely covered by antheridia, even in the rather young condition, and this fact makes it very unlikely that we had another specimen of *S. mixta*. The antheridia were both diclinous and androgynous.

The material was obtained from a jar containing Elodea, and brought from the Huron river in September. A culture from a single zoospore was obtained January 10. The series of cultures were similar to those used with the preceding species. Haemoglobin with the various salts always produced abundant gemmae, which were usually large, fusiform or spherical in shape. With rubidium hydroxide no gemmae appeared; only a luxuriant vegetative growth seemed possible. With leucin and the series of salts the results were practically the same as in the haemoglobin cultures.

K. In contrast to the two preceding forms, this species produced sporangia in abundance. These sporanges were often clavate to narrowly ovate, and were arranged irregularly in acropetal series. This indicates a *Pithyopsis*. No oogonia were seen in any of the cultures, although entire series of both haemoglobin and leucin cultures were carried through. As in the case of the two preceding forms, gemmae were present in all of the cultures except in that of rubidium hydroxide; in the latter

the vegetative appearance was excellent. The gemmae in these cultures often passed over into zoospore formation in great numbers. Their shape approximated furthermore closer to sporanges than to oogonial initials.

M. *Aphanomyces laevis* was also obtained in pure culture from a single zoospore, but only a few cultures could be made at the time.

General Discussion.

The different phases of the investigation may be divided into the following headings:—

I. Relation of growth and reproduction.
II. The development of the sexual organs.
III. Physiological varieties.
IV. The problem of species.

I.

All the species studied above showed the same general reaction on the various substrata as *Saprolegnia mixta*. They all grew rapidly on beef-gelatine, meat, and pea-broth. There is, however, a decided difference in the rate of growth; and on this point some observations were made with beef-gelatine cultures in Petrie dishes. Cultures of *S. mixta* (H), *S. hypogyna* (C), *S. mixta* (F), of the three unknown species (I), (K), and (L), and of two species of *Achlya*, were made on beef-gelatine by inoculating the centre of the dishes with them. The cultures were started February 13, and on the 19th (I), (K), and (L) had grown to the margin of the dishes, while (H) and (F) and the two Achlyas were just half way; (C) had reached a point about half way between the other two lots. The dishes were all kept under the same conditions of temperature, &c. Other incidental observations had all along pointed to this result. This of course does not indicate the absolute rate of growth. Under favourable conditions of temperature and nutrition they grow to the margin in a very much shorter time. The few trials made indicate a rate of ·2 mm. per minute, or even less than a minute.

It is to be noted that the two rates of growth are correlative with the two sets of species as regards their tendency to produce reproductive organs. (I), (K), and (L) do not produce oogonia under the conditions found by Klebs for *S. mixta*, or under the same conditions as found by myself for *S. hypogyna* and (H) and (F). This indicates a close physiological relationship between these latter species. It is quite clear that (I), (K), and (L) are different species, and if they are Saprolegnias, that they are possibly of close relationship among themselves. On the other hand (H), (F), and (C) seem to be also closely related physiologically, and a study of the nutrition relations of other species like *S. monoica*, *S. ferax*, and *S. torulosa* may show that these, too, act like *S. mixta*; such results would give us not only a morphological but also a physiological basis for classing them

together as the 'ferax' group. Maurizio also found that many species of *Saprolegnia* which he met produced no oogonia, but he only tested them with ordinary substrata.

(H), (F), and (C) were kept vegetative for six months at a time by continued cultivation on beef-gelatine. It was found best to transfer from the old to the fresh plate as soon as the mycelium had reached the margin of the dish, as it was noticed that when the culture was allowed to get too old the transferred hyphae no longer grew. This was either due to the accumulation of waste toxic substances produced by metabolism, or to famishing on account of the inability of the hyphae to get at the gelatine, the substratum immediately surrounding them being rapidly dissolved by the proteolytic enzyme secreted by the fungus. In order to determine this point a culture of *S. hypogyna* was made in a flask containing 500 cc. of a 5 per cent. maltose and a 0·1 per cent. peptone solution. The mycelium rapidly filled the liquid, forming a thick mat near the surface and remaining in a vegetative condition *for thirteen months*. The mass was then taken out, carefully examined, and a new culture made from it on beef-gelatine. After a time it was tested and found to produce the same reactions of *S. hypogyna* already recorded for that species. The mycelium from the thirteen-month culture was found to be in part disintegrated, in part filled with a very thin protoplasmic content somewhat granular in appearance, while a number of the end hyphae appeared normal. These apparently still retained the necessary vigour for further growth on a favourable substratum. No reproductive hyphae could be found either in the loosely wefted peripheral zone of the mass nor in the interior. Vegetation therefore took place for thirteen months without the renewal of the substratum and in spite of the chemical substances excreted during the metabolic activities of growth. This is possible because, as Klebs has shown, the metabolic products of a carbohydrate substratum are much less harmful than those of a proteid substratum. The latter is always found unfavourable for the continued existence of the fungus because of the toxic character of the waste products excreted. The small amount of peptone used supplied the necessary nitrogen, without interfering with the life of the fungus.

The proposition of Klebs, now pretty well accepted, that the fungus can be kept indefinitely in a vegetative state, seems to be well founded. Klebs himself kept *S. mixta* in a vegetative condition for six years, and all the species studied by me were kept at least six months without producing zoospores, oogonia, or gemmae of any kind; and, as just seen, *S. hypogyna*, which can be made to reproduce most abundantly within a few hours or days, was kept from doing so for thirteen months; at the end of that time it was able to produce oogonia in three days.

Although it seemed superfluous to repeat all of Klebs's experiments, a set of cultures was prepared to study the effect of the different concentra-

tions in order to find the optimum concentration for the production of oospores. The substances selected were haemoglobin, peptone, glycocoll, urea, dextrose, and fructose. The results obtained, both as to amount of growth under different concentrations and the time required for the formation of the reproductive organs of both kinds, approximate so closely to his results that it was decided to use his optimum concentrations for the further studies.

II.

The effect of nutrition on the development of the sexual organs was first effectively studied by Klebs. He also pointed out a significant variation in the number of antheridia present under different conditions. *Saprolegnia mixta*, studied by him, was said by De Bary to have normally about 50 per cent. antheridia, which according to Trow are functional male organs. Klebs found that in a pure leucin culture—apparently 0·1 per cent. solution—no antheridia appeared ; but on the addition of Knopp's solution of inorganic salts, or of a mixture containing K_3PO_4, KNO_3, and $MgSO_4$, the antheridia were formed in considerable numbers. He then tried various inorganic salts with 0·1 per cent. leucin, and found that K_3PO_4, K_2HPO_4, and KH_2PO_4 caused the number of antheridia to be increased, potassium phosphate being the most effective and producing them on 50 per cent. of the oogonia.

The form of *S. mixta* used by me seems to deviate in some of these respects. The normal fly culture of (H) has as high as 75 per cent. antheridia on its oogonia. This is also the case in a 0·1 per cent. leucin solution. It is also seen that in my cultures $Ca(NO_3)_2$, $Ca_3(PO_4)_2$, and $MgSO_4$ acted so favourably on antheridial formation as to produce a yield of as high as 90 per cent. or even more antheridia-bearing oogonia. Not only were there no antheridia found in the sodium and ammonium phosphate solutions, but even the oogonia were lacking. These latter salts, then, act as did those in Klebs's solutions, and inhibit in some way under the conditions the formation of oogonia.

If we now compare the results obtained in *S. hypogyna*, the effects of inorganic salts in inducing antheridial growth is shown markedly. Pringsheim, as a result of his belief that parthenogenesis is brought about by changes in the external conditions, considered the plant in question as merely a variety of *S. ferax*. De Bary, however, considered it a valid species, on the basis of a culture which he kept for three years, and in which, he says, it remained constant, not only with reference to the hypogynous antheridial cell so characteristic of the species, but also as to the persistent absence of antheridial filaments.

It was my original intention to study *Saprolegnia ferax*, which is said to lack the antheridia entirely ; whether it does so or not has to be settled by a culture from a single spore. As this species did not appear in my cultures

at the time when I needed it, the study has not been made. There appeared, on the other hand, a *Saprolegnia* which I discovered to be *S. hypogyna* as detailed above. As this species seemed to offer as great, if not greater, possibilities than even *S. ferax*, it was decided to study it instead.

A pure culture was transferred to a sterilized wasp as offering the ordinary insect culture-media in general use. As in *S. mixta*, sporanges are formed and emptied in great numbers within a few days and following a luxurious growth of hyphae. On the ninth day oogonia were abundant, and a few days later very abundant, containing from one to ten oospores. In this culture not a single antheridial filament was found, although it was diligently sought; also the hypogynous cell was always present, and all the other chief characters of the plant agreed with the description by De Bary of *S. hypogyna*. If, therefore, Pringsheim and De Bary had the same species which I had, and it would seem that they had at least one of the forms, then, as long as they used flies or similar substrata, they would be sure to find only the hypogynous cell and no antheridial side-branches.

Now if *S. hypogyna* is grown in ·05 per cent. haemoglobin solution, its appearance is nearly like that on the wasp, except that a very few hypogynous cells have a short protuberance—an abortive antheridial filament. If peptone is added to the haemoglobin solution no protuberances appear at all. In haemoglobin solutions Klebs found that K_3PO_4, Na_2HPO_4, and $Ca_3(PO_4)_2$ were the only salts which induced antheridial formation in *S. mixta*. As noted in another part of this paper, not only these but other salts also induced antheridial formation in the case of *S. hypogyna*. These different inorganic salts act with very different degrees of effectiveness; in fact it is possible to select a regularly graded series of cultures showing the relative value for antheridial formation of the several salts used. Such a series is shown in Plate XXIII. The haemoglobin cannot be sterilized, but as the cultures were made in winter and kept continuously at a temperature of about 15° C., no trouble was experienced in this respect. The haemoglobin used (Merck's) was very clean itself, and if the paper, dishes, &c., used in weighing and transferring were sterilized, very seldom did any bacterial contamination appear in the comparatively short time necessary for the experiments. The following salts, decreasing in effectiveness for the production of antheridial side-branches, were used: K_3PO_4, KNO_3, Na_2HPO_4, K_2SO_4, $(Ca_3PO_4)_2$, and $Ca(NO_3)_2$, and these were added to a haemoglobin solution. It can be seen that *S. hypogyna* is very well adapted to show off the action of the various salts. KNO_3 and K_2SO_4 were especially favourable and produced antheridial branches on as high as 40 per cent. of the oogonia. This result differs somewhat from that of Klebs, and the question of impurities in the salts used would naturally arise. It must be remembered, however, that minute amounts, such as might be present in the salts used, do not affect the production of oogonia and antheridia, at least not to such a

striking extent. The salts were obtained from Kahlbaum and had the C.P. stamp. As to the rest, the cultures were always made in series, so that the checks would show any outside influence. With leucin, as noted, $(Ca_3PO_4)_2$ and $MgSO_4$ also caused the appearance of antheridial side-branches, and it might seem from the action of so many salts that it was due to the effect of inorganic salts in general. To get some light as to the possible effect of concentration, the following was tried: $Ca_3(PO_4)_2$, in the concentration of 0·1 per cent., yielded antheridial side-branches very rarely in haemoglobin solutions. Hence a concentration half as great was used, but with no marked result. There is room, however, for further experimentation along this line.

If we now examine the effect of the different salts in detail, we find that certain differences stand out in a pronounced manner. In a normal culture on insects or haemoglobin the usually terminal oogonium is globular, sometimes flask-shaped, the smooth wall rather firm and with rather large pits which do not project above the surface (Figs. 3 and 12). The oospores are from one to ten in number, sometimes more, and always centric in structure. Attached to the base of the oogonium is the hypogynous cell, cut off from the rest of the oogonial filament by a cross-wall, and in length usually from half to once the diameter of the oogonium. Normally this cell is filled with protoplasm, and finger-like projections often extend from the upper end into the oogonium; at times there is merely an upward bulging of the wall separating oogonium and hypogynous cell.

In cultures of haemoglobin and peptone (Figs. 1, 2, and 3) the pits become quite prominent at times, as if the turgor of the maturing oogonium had pushed out the thin-walled pits.

In calcium nitrate solutions, the cross-wall separating the hypogynous cell from the oogonial stalk may be entirely lacking; when present it occasionally produces short side-branches, but these never differentiate any antheridia. Oogonia are formed in great abundance with a great many oospores, and the whole organ has a very healthy appearance, while the oospores matured in every oogonium during the period of examination. The pits vary and may be large or small.

In calcium phosphate cultures (Figs. 8, 9, and 10), there is produced in the oogonial filament more than one cross-wall, at times three cells being cut off in this way. This was also observed in the cultures with calcium nitrate, as well as in some of the potassium nitrate cultures. Just what the stimulus is in the formation of these extra cross-walls is not very clear. Molisch ('95) found calcium necessary for wall-formation in *Spirogyra*, and this may also be a factor here. It is well known, on the other hand, that the mycelium of some Saprolegniaceae can form cross-walls under poor nutrient conditions, but this never occurred as far as my observations went in this species. Maurizio ('95) considered this doubling and tripling of cross-

walls as a character to distinguish, along with the size of the oospore, one of his varieties.

The most potent solutions for inducing antheridial formation are the solutions containing potassium phosphate and disodium phosphate (Figs. 11, 13, 14, 15, 16). In solutions with potassium phosphate the antheridial filaments develop completely, i. e. the antheridium is differentiated at the apex of the side-branch, and is applied in the normal manner to the oogonium, and fertilizing tubes are put forth and touch the oospheres. Such a case is seen in Figs. 16 and 13. We have here, then, a combination of conditions much more favourable than in the preceding cases. It is seen, too, that there is considerable variation under these conditions with reference to the source of the antheridial side-branch. If the hypogynous cell is considered as a potential antheridium, we would expect that the side-branch would naturally arise from it. This is not always the case; instead, the side-branch may arise from below the hypogynous cell, as seen in Figs. 11 and 13; or it may arise from the oogonial branch immediately below the oogonium in the absence of the hypogynous cell, as in Fig. 14 *a*. And, finally, as shown in the same figure, *b*, it may arise from a different filament in the usual manner of antheridia of diclinous origin. No cases were seen where the antheridial filament or branch grew out of the oogonial wall as in *Achlya racemosa*.

The question of whether the tendency of antheridial filaments to arise from the oogonial stalk or from some other branch of the fungus is of sufficient constancy to warrant its use as a specific character seems to be answered here (Fig. 14). There is no doubt that the tendency of the male filament to arise at a definite point is to a large extent a matter of conditions. In *S. mixta* (H), which I studied, the oogonia were located either apically on short branches, or on the ends of long filaments, or intercalary. On short-stalked oogonia, the antheridial filaments grew from both the same and other filaments, i. e. were both androgynous or diclinal; the oogonia on the ends of long filaments usually were supplied by diclinal antheridia, and the intercalary entirely by the latter. Evidently the position of the oogonia on the plant conditioned here in some way the origin of the antheridial stalk. In *S. hypogyna* this was brought out strikingly in the cultures with K_3PO_4, and less so in those with KNO_3 and Na_2HPO_4. Here numerous oogonia occur in which the usual hypogynous cell is present, but there are no antheridial filaments developed from any of them, nor from the oogonial stalk anywhere; on the other hand, the oogonia are surrounded often very profusely by antheridial filaments with well differentiated antheridia, as shown in Fig. 18. Here, then, all the antheridia are of diclinous origin, and the fact that my cultures were made from a single zoospore goes far to establish the notion that the place of origin of the antheridial filament cannot be retained as a good specific character, at

least in ordinary variable cultures. It is also to be noted, that in all the species of *Saprolegnia* which I studied from a single zoospore, and in which oogonia and antheridia formed at all, the antheridial filaments were of both androgynous and diclinal origin, or would become so under the varying conditions of culture. Whether this variability extends to all the species of *Saprolegnia* and of *Achlya* also, cannot be determined in the absence of experimental evidence.

The results so far indicated show *that it is possible to produce, where hitherto they were believed to be absent, antheridial branches of the normal type known in other species of the family, and that their production is conditioned by the presence of definite inorganic salts.* It seems clear, also, that more radicals of salts subserve this function than was found to be the case in the *S. mixta* studied by Klebs. At first sight, calcium might also seem to be necessary; but no results were obtained with any other calcium salts than $Ca(NO_3)_2$, $Ca_3(PO_4)_2$, and $CaHPO_4$, and this would seem to indicate that nitrogen and phosphorus are the active elements. This view is strengthened by the fact that the most active salts are K_3PO_4 and KNO_3. But we have seen that K_2SO_4 also belongs to the list. From the data so far at hand it is likely that potassium is therefore also an active element. This is also apparent in the cultures where K_2SO_4 was added to the solution of Na_2HPO_4. When the latter alone was used with haemoglobin the development of reproductive organs was not near as definite as with K_2SO_4. In any case side-branches were produced and developed to a greater or less extent in all these salts when used separately with haemoglobin.

Just what we mean when we say that one element is more active than another is not yet clear. Our knowledge of the process or sets of processes which start the mechanism of antheridial formation going is practically nil. We can with De Bary ('81) take the view that a chemical compound is excreted by the oogonia which induces the formation of antheridial filaments, and that the different salts offer the conditions which make the secretion of this active principle possible. Klebs found only the phosphates especially effective for the induction of antheridia; but if it is true that nitrates and potassium are also active, then the problem becomes still more complicated. If it depends in its entirety on the improved condition of nutrition, as Klebs believes, then the effects of phosphates, nitrates, and potassium is more easily understood, although it still fails to afford any explanation of the actual relations of antheridial filament and mycelium. That the relations are chemical in nature is made plausible by the researches of Pfeffer ('84) and Miyoshi. It would be conceivable that some constituent of the salts used combines within the cell in such a way with the proteids as to set free an enzyme, and that this is the active principle. The origin at so many different points indicates that it is largely a matter of equilibrium of the conditions which do take part, and that the equilibrium, or perhaps lack of

equilibrium, has occurred at the point where the antheridial filament originates.

In this connexion attention should be called to Figs. 17 and 18, which show a condition apparently seen in part by Klebs ('99, p. 566). Here the antheridial filaments, poorly developed on the oogonium of Fig. 17, but with well-developed antheridia in Fig. 18, grow profusely around the oogonia. In all these cases, however, the oospheres seldom mature, but disintegrate as if absorbed. Klebs indeed states that antheridia-like filaments form in profusion, but, in his opinion, instead of fertilizing the oospheres, consume them. From his brief statement it seems that he did not observe definite antheridia at the apices of the filaments in his *S. mixta.* As shown in my figure, these are very abundant and definite in such cultures of *S. hypogyna.* That these are true antheridial branches is shown by these antheridia at the apices as well as by the presence of intermediate forms where the short side-branch has developed to a greater or less extent in the direction of the extreme stage. They are mostly without fertilizing tubes and lie only on the surface of the oogonium; the figure lacks perspective, and the antheridia, which seem to have penetrated the oogonial wall, lie on its hither surface. The idea that the antheridia devour the contents of the oogonium is hardly applicable in this case; and yet there seems to be a close connexion between the disintegrating process and the presence of many antheridia. In Fig. 17 two oospores were formed before the blighting effect of the male filaments reached them, while the remaining oospheres disintegrate. It may be that this phenomenon is explicable in another way than by assuming a direct evil effect due to contact of antheridial filaments, viz. that the nutrition the plant as a whole is directing into these filaments is taken from the amount which would normally supply the oogonial filaments, and hence the latter are famished. There is, however, the difficulty that one finds in the same cultures with these extreme forms, oogonia with male branches well developed, and which have the normal structure as in Fig. 16.

As already stated, not only was the effect of phosphates very marked, but also that of nitrates, and even potassium sulphate and magnesium sulphate showed a favourable increase in the number of oogonia as well as of antheridia. To test the stimulative effect of salts whose nutritive value is known to be of no importance in certain other fungi, sodium chloride was added to a number of haemoglobin solutions along with potassium phosphate, and compared with those containing phosphate and haemoglobin only. There was a decided improvement in the appearance of the oogonia, as well as in the number of antheridia formed. Fig. 18 was drawn from an example of such a culture. A set of cultures with potassium sulphate added to the other ingredients seemed also to have a favourable effect, perhaps neutralizing the disintegrating effect of K_3PO_4, and allowing the oogonia

to reach maturity by inhibiting the production of profuse antheridial filaments.

A further result that requires notice is the effect of rubidium and caesium hydroxides. These were added to the haemoglobin solutions, the same as the other salts. The results in all the cultures was a good vegetative growth and a complete inhibition of sexual organs; and not only these, but the sporanges, and even the gemmae, were almost entirely prevented forming. Even after two weeks in a 0·1 per cent. solution with haemoglobin only vegetative hyphae were present. Since rubidium was found by Benecke ('95) to be able to replace potassium as an essential element to growth, it was to be expected that it could replace it in the rôle of an aid to the formation of sexual organs. Of course it may be that potassium does not, as such, affect the production of oogonia to any great extent. The rubidium was not tried in the form of a nitrate. It is possible, too, that, like sodium, potassium merely performs the function of accelerating the process of reproduction; but, if so, one might still expect the same property in rubidium. The view that the sexual organs are produced only after a good growth of mycelium would seem at first thought to find an exception in the case of rubidium, and in order to avoid that conclusion one must consider it as acting inhibitive in such cultures.

III.

The observation that certain species of the Saprolegniaceae never or seldom develop sexual organs has been made before. The subfamily Leptomitae, as used by Humphrey, includes only one species of *Apodachlya* which develops sexual organs, a new species described by Humphrey himself. Maurizio ('96) found 'many species of *Saprolegnia* which yielded no oogonia even after three to six months of culture'. I obtained from five different sources, two of which are not mentioned in the introduction, species from single zoospores which either formed no oogonia at all, or, in one case (I), only a few. One of these was presumably a *Pithyopsis*, although no final decision was reached as to its position. No oogonia were seen at all, either on this or on (L), in any of the cultures which were set up especially to determine this point. These species vegetated very luxuriantly, and just whether they were all distinct species or not is uncertain; the same rate of growth and the sporanges are the only evidence to go by. We have here, however, a distinct physiological character by which these species of *Saprolegnia* are separable from those that produce oogonia so readily, like *S. ferax*, *S. mixta*, and *S. hypogyna*. The question here naturally arises whether these plants ever produce oogonia. I am inclined to think that they do, and that the conditions necessary are merely of a different kind than those so far tried on them.

Klebs found that different species of *Oedogonium* behaved very differently to a change from light to darkness in the effect this change had on the production of zoospores. I have been able to verify this for several species of *Oedogonium* in our laboratory. It is therefore not surprising to find similar differences of a deep-seated nature present in the various species of *Saprolegnia*. We need further experiments with these forms in order to determine the conditions.

The two forms of *Saprolegnia* which I have denominated (H) and (F) need further discussion. In all the essential characters except the number of antheridia these two forms would come under *Saprolegnia mixta*. (H) comes more nearly to it except that about 70 per cent. oogonia have normally antheridia, and this can be increased to over 90 per cent. This form approaches *S. monoica* in its many antheridia, and it is very probable that under the most advantageous conditions all the oogonia would bear antheridia. But *S. monoica* has been described as having only androgynous antheridia, larger than those of *S. mixta*, and with long and stout vegetative hyphae. On the basis of what we have learned concerning the variation of antheridial filaments of *S. hypogyna*, it would seem that the androgynous origin of antheridia was not likely to be constant enough to warrant keeping two species separate on its account. It seems to me, however, that this is too large a conclusion. For, although no genuine *S. monoica* was found in my cultures, it seems likely from Humphrey's careful observations that the size of the hyphae and antheridia, as well as the usually 'indescribable differences', make this an easily recognizable species; Humphrey also points out that it is comparatively rare. I can, therefore, not subscribe to the suggestion made by Davis ('04) that *S. mixta* is only a cultural form of *S. monoica* or vice versa. But neither does my plant (H) seem to be exactly the form described by De Bary, since both he and Klebs found that 50 per cent. was the highest number of oogonia which were supplied with antheridia.

This would hardly be a point on which to insist, if the results from my study of (F) did not also warrant it. The cultures of (H) and (F) were made side by side, and constitute checks on each other with reference to temperature, &c., so that the uniform differences must be considered constant. Each was derived from a single zoospore, and the cultures were kept from becoming contaminated. When the cultures were made on wasps, (F) had from 1 to 2 per cent. antheridial oogonia, while those of (H) amounted to 75 per cent. or more; in leucin solutions those of (F) gave a maximum of 25 per cent., while those of (H) yielded as high as 95 per cent. of antheridial-bearing oogonia. It seems to me that we must consider these as separate entities which are closely related to the *Saprolegnia mixta* of De Bary, and that they will remain distinct along with that species when all are placed under the same conditions of culture. Nor does it seem to me

that the results of Klebs, Davis, and myself on the variability of *Saprolegnia mixta* or of any of its forms, in obtaining cultures which produce no antheridia, warrant the view that this condition represents *Saprolegnia ferax.* This species, it is true, is said to have no antheridia; but any one who has seen the true *S. ferax* (*S. Thureti*) will remember some very essential characters which always stand out in fly cultures of this species. One of these is the presence of large cylindrical oogonia with the oospores in a single row. Humphrey has expressed himself forcibly on this point: 'The rather common occurrence of cylindrical oogonia and the very conspicuous pitting of the oogonia mark this species unmistakably.'

With reference to the variability of *Saprolegnia hypogyna* there are several points that need attention here. I have already fully indicated some lines of variation. Now if these variations could be fixed, the resulting forms would be similar to the varieties of *S. hypogyna* described and figured by Maurizio ('94). According to him, all his varieties agree with De Bary's *S. hypogyna* in the following characters: firstly, the hypogynous antheridial cell; secondly, the centric oospheres; thirdly, the flask-shaped oogonia. The oogonia of my plant were globular, which according to De Bary's original description ('88), is one of the shapes. In his 'variety I' the antheridial cross-walls are nearly always wanting—a phenomenon also noticed in some of my cultures—the oogonial stalk is variously bent, and the pits project somewhat above the surface of the oogonium—also noted in my cultures. In his 'variety II' the characters are: many pits, straight stalk, and additional cross-walls on the oogonial stalk. In my cultures the pits were found to vary in number and size, and additional cross-walls on the oogonial branch were common in calcium phosphate solution, as shown in Fig. 9. His 'variety III' is like his II except the additional cross-walls, and in its short protuberances on the side of the hypogynous cell.

Here, then, is a form found in nature with all the characters of those forms which I succeeded in getting in cultures with $CaHPO_4$, as shown in Figs. 5, 6, and 7 (compare also Fig. 20, Pls. IV and V, Maurizio, '94). In both the side-branches have attained only partial development, as no antheridia are cut off from them. The hypogynous cell is usually empty, as is also the tube which pushes up into the oogonial wall in some cases; in fact so much alike are his 'variety III' and my cultural form that it is difficult to select differences, the number and size of pits seeming to be the only differing character.

Maurizio's 'varieties IV and V' are characterized by the few pits; the former also by the thin oogonial wall, and the latter by the presence of definite protoplasm within the projection of the hypogynous cell where it pushes into the oogone; both lack side-branches. No especially thin walls were noticed in the oogonia of my cultures. The presence or absence of a healthy-looking protoplasmic content of the projection certainly varied

in my cultures to the full extent of what Maurizio observed. Sometimes the hypogynous cell itself is transparent; again, they may be filled with protoplasm; the last condition is especially marked in calcium nitrate cultures.

The comparisons just made serve to bring out clearly the possibility of inducing a variation which overlaps or imitates a character in a form which is apparently constant in nature; and this fact naturally leads to a consideration as to the meaning and values of varieties and species, a theoretical question that may better be discussed in a separate section.

The significance of the hypogynous cell has been fully discussed by Maurizio (loc. cit., p. 149), and my own results seem to bear out his contention that the projection into the oogonium is not a fertilization tube, but represents merely a tendency in this genus to produce secondary growth, as in sporanges. It was often noticed that, even where a side-branch was fully developed and bore an antheridium of its own, these projections appeared to push up to a greater or less extent. Probably the hypogynous cell itself is no true antheridium, but merely an aborted or latent cell which has either lost its sexual function under ordinary conditions or had never attained it; in either case we would hardly expect that its function could be so easily called forth as to form these projections as fertilizing tubes. Furthermore, it is not rarely absent altogether, and then the ordinary vegetative filament is the starting-point of the antheridial stalk.

IV.

There are several theoretical questions which this investigation touches and which may be briefly discussed. The first of these is the question of the constancy of species. As De Vries has pointed out, experimental evidence on this point is not very extensive. The expression has been used for a long time to indicate a constancy of characters during culture, viz. that if the individual remained constant in its specific characters it proved the autonomy of the species, while if it varied in a certain direction it belonged to another species, and the direction of variation indicated the species to which it belonged. Such cultures were usually made under so-called natural conditions. If, for example, a specimen of *Saprolegnia* was grown on a fly for three years and showed no change of a retrogressive or other nature in the characters to be tested, it was declared a definite species. If, on the other hand, the new characters disappeared at times, or were changed during the course of the cultivation to a character already known, the new form was considered merely a variety.

This is the attitude at present exemplified by mycologists who describe new species of the higher fungi. Often a third name is added to the binomial, and this implies that the new characters of the plant in question

are so few or of such a grade of importance as to leave open the question of its rank. Later specimens of what seem the same fungus are received from other sources, and if all the new characters are also present in these, the evidence is considered sufficient to raise the fungus in question from its varietal position to the rank of a new species.

This attitude fails to take into account one important consideration, viz. the question of the Constancy of Conditions under which these different individuals grew, or under which their constancy of character was tested. If, as has been brought out by recent investigators (Klebs, '06), the same conditions of nutrition, temperature, &c., produce always the same result, then the mere cultivation for a long period of time in and of itself is no test of species in the sense in which the term has been used. The problems of constancy include a trial of all the conditions. It would seem that we are driven to the same resources for accuracy in biological studies which are fundamental in physics and chemistry, namely, that of standards. Each species is recognizable, not only by certain prescribed or described characters, but in addition by these characters only when present under certain definite prescribed conditions. For example, *Saprolegnia hypogyna* is known by the hypogynous cell on the oogonial stalk and by certain other less important characters, all of which are present under the conditions of culture on a fresh fly, or more precisely in a definite per cent. leucin or haemoglobin solution, at a temperature, say, of 20° C., &c. Differently stated, this implies that the characters acquired in a new environment are persistent as long as the environment is persistent. It would seem necessary, then, in monographing such a family as the Saprolegniaceae to refer all the species to definite conditions, which should be uniform for all, and to determine in each case the variability to the extreme attainable limits; we should then have attached to each description of a species an account of its variabilities. This view demands that comparisons with old descriptions should take into account the exact condition under which the plants were raised; a procedure fraught, no doubt, with difficulties. Such a physiological basis for species seems to me the only starting-point in a revision of the relationship of the species of any family of plants. Whether it is practicable in all cases is another matter which cannot be discussed here.

Another question which always arises in this connexion is the one as to whether species are distinct or whether there is a gradual blending of one species into another by means of intermediate shadings. In other words, whether we have new species formed by mutation or by continuous variation. It is not my intention to take sides. I merely wish to point out in what way my results touch upon the problem. As has been seen, some of the forms of *Saprolegnia* are very close to each other morphologically, and yet are physiologically distinct. This appears very strikingly in such forms of *S. mixta* as (H) and (F), along with the one that Klebs

studied. Although the number of antheridia-bearing oogonia seem the chief constant distinctions, other minor and less noticeable differences are present. Maurizio made out a series of six or more forms, with constant differences, of *S. hypogyna*. It is not certain that his conditions of culture were uniform for all his studies, for Maurizio worked under the assumption that time was the principal factor in the test for constancy. The fact that under certain conditions I was able to obtain various characters which distinguished his varieties, seems to indicate the method by which Maurizio's varieties may have been derived; namely, by mutations from the simplest *S. hypogyna*. It is necessary to distinguish clearly between physiological variation and mutation, but this need not preclude the possibility of a mutation taking place in a direction such as a physiological variation takes. Of course the whole problem becomes deeply involved because of our complete ignorance as to what conditions a mutation.

It seems to me conceivable that the variations of any individual may extend far in any one direction; and also that the variations may be such as to produce structures which are present in other bona fide species, and yet the two species need not necessarily be phylogenetically related. In this way we would expect to find overlapping of forms, and it is on this supposition that the possibility rests that Maurizio's plant is not the same as that obtained by me in $CaHPO_4$ solutions and apparently having all the characters of his variety.

What shall we call all these closely related forms? De Vries has offered to solve the problem for us in the term 'elementary species', with all that it implies. Because of the presence of parthenogenesis in our plants, and in the absence of cultures carried through generation after generation from oogonia, no evidence in this paper can be offered to substantiate or to refute De Vries's view. But indirect application of his theory to the facts obtained in the foregoing pages is possible, and to that extent only has it been applied in the foregoing pages. Klebs's notion of variations, on the other hand, is strictly substantiated in so far as it applies to the individuals studied. Furthermore, his idea that we can produce De Vries's retrogressive and degressive mutations by the change in conditions seems also borne out by the results. Shall we say, then, that the only mutation which exists is progressive mutation, and that the many forms found, and which remain constant from each other under the same conditions, were developed from some common ancestor by this method? In so far as the matter of this paper is concerned, this would be an apparent explanation of the facts. Whether it is the real one cannot be determined by such few and narrowly restricted experiments.

Summary of Results.

(1) It is possible, by the method of culture outlined, to isolate species of *Saprolegnia* relatively quickly by means of a single zoospore.

(2) The study of the various species of *Saprolegnia* verifies the work of Klebs on *S. mixta* as to the effect of nutrition on the differentiation of the reproductive and vegetative processes.

(3) Not all the species of *Saprolegnia* produce sexual organs under the conditions favourable for those of *S. mixta* and *S. hypogyna* and are therefore physiologically as well as morphologically distinct.

(4) *Saprolegnia hypogyna*, which has morphologically no true antheridia, can be made to develop such under proper nutrient conditions; K_3PO_4, KNO_3, Na_2HPO_4, $Ca_3(PO_4)_2$, and $Ca(NO_3)_2$, added to haemoglobin solutions, are, in different degree, effective to this end.

(5) The antheridial filaments arise in either a diclinous or an androgynous manner in *all* the species examined under varying culture conditions, and hence their origin is of no special importance as a diagnostic character. No sign of heterothallic species was detected, unless the absence of sexual organs in some species points that way. It is believed that these species have not yet been placed under the proper conditions for the formation of sexual organs.

(6) The variations were so extensive that nearly all the characters used for diagnostic purposes in this genus were affected. At first thought this seems to indicate that no separation can be made of the different species of *Saprolegnia* by means of constant characters. We can, however, use the resources of the chemist and physicist, and, by stating the exact conditions of culture, establish a standard to which all forms may be referred. A species thus defined can be identified by placing it in these conditions. The assumption here needed seems to be perfectly justified; for it has not been proved as yet that characters which have been developed under cultivation will be retained by the plant when it is returned to the original environment.

(7) In spite of the great similarities of certain very constant forms and their overlapping under different conditions, it is concluded that they are entirely distinct forms, at least physiologically, if not always morphologically. This result, combined with what Maurizio and De Bary found in this family of fungi, forces one to assume that there are a great many simple forms—elementary species in the sense of De Vries—within the genus *Saprolegnia*.

In conclusion, I may say that this paper adds something more of evidence towards the doctrine that sex in plants is determinable by external conditions. It is admitted that the evidence is not conclusive; for the structure and the development of the plants studied are of such a nature as

to preclude any final results on this point. If we could start with a dioecious form of *Saprolegnia* and obtain the results here obtained, the outlook would be more encouraging. Of course if we were to consider that the normal condition of *Saprolegnia hypogyna* is one in which the antheridium is simply suppressed, and that the new conditions have merely removed the obstacle which had hitherto prevented its development, sex-determination would not be touched by the reactions detailed in this paper. But I am not at all sure that the hypogynous cell must be so considered. At any rate, the question of sex-determination remains one of the most attractive problems of biology.

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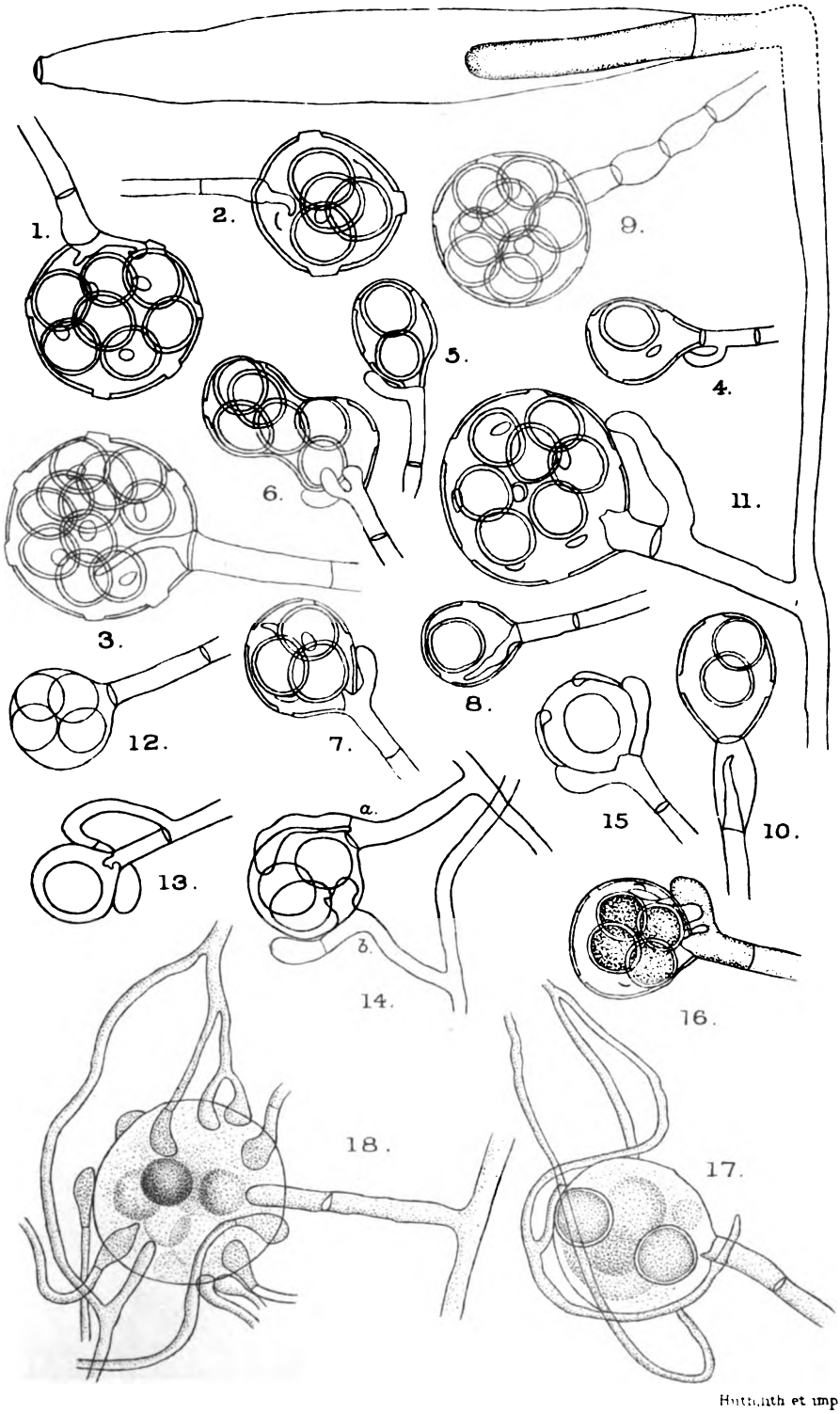
EXPLANATION OF FIGURES IN PLATE XXIII.

Illustrating Dr. Kauffman's Paper on Saprolegniaceae.

All the figures were drawn with camera lucida. They are all taken from specimens of *Saprolegnia hypogyna*. The magnification is uniform for all the figures.

Figs. 1–3. From cultures in haemoglobin and peptone.
Figs. 4–7. From cultures in haemoglobin and $CaHPO_4$.
Figs. 8–10. From cultures in haemoglobin and $Ca_3(PO_4)_2$.
Fig. 11. From cultures in haemoglobin and KNO_3.
Figs. 12–15. From cultures in haemoglobin and K_3PO_4.
Figs. 16–18. From a culture in haemoglobin and K_3PO_4, with an addition of NaCl.

UNIVERSITY OF MICHIGAN.
May, 1907.



Huth,lith et imp

KAUFFMAN—SAPROLEGNIACEAE.

The Genus Endocalyx, Berkeley and Broome.

BY

T. PETCH, B.Sc., B.A.

Mycologist to the Government of Ceylon.

With Plate XXIV.

THE genus *Endocalyx* was established in 1875 by Berkeley and Broome for the reception of two 'species' sent from Ceylon by Thwaites. They characterize it as 'peridium calyciforme, pedunculatum, villosum, demum ruptum, e basi crassa oriundum; sporae subglobosae echinulatae,' and place it in the Myxomycetes, with the remark: 'This curious genus is evidently closely allied to *Alwisia*. The spores, however, are very different and not half the diameter.' Saccardo lists it among the doubtful genera, and it is not referred to by any of the compilers of Engler-Prantl, Pflanzenfamilien. Mr. Lister does not refer to it in his monograph of the Mycetozoa.

The genus does not appear to have been identified since, and apparently the type-specimens have not been re-examined. Part, at least, of Thwaites's gathering is in the Peradeniya herbarium, and an examination of this shows at once that it does not belong to the Mycetozoa, and that the two species are identical: they were, in fact, based on an incorrect separation of one of Thwaites's numbers, a frequent occurrence in the examination of his consignments. The resemblance to *Alwisia* is not very evident, and what resemblance there is is purely superficial: the spores are three to four times the length of those of *Alwisia*.

Although I have not succeeded in collecting fresh specimens of this species, two other members of the same genus have been found in the Peradeniya Gardens, and by the help of these it is possible to interpret the herbarium specimens of *Endocalyx Thwaitesii*, B. & Br., and *Endocalyx psilostoma*, B. & Br., both of which were founded on damaged specimens. One of these forms does not appear to have been described before; the other occurs most abundantly on all dead palms, and has been named and described from almost every tropical country; but as its structure could never be accurately made out from the preserved specimens the descriptions have never been recognized by later workers. No one who collected Fungi in the Tropics and included the smaller forms in his collection could fail to

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gather this second species, but it is so fragile that it could never reach the describer in an undamaged condition. It was sent by Thwaites to Berkeley and Broome in the same collection as the specimens they named *Endocalyx*, but they failed to recognize that it was at all related to the latter, and named it *Melanconium melanoxanthum*. Cesati gave the same name to specimens collected by Beccari at Peradeniya, but all recent describers appear to have overlooked the fact that it had been named before.

ENDOCALYX MELANOXANTHUS (B. & Br.)

(*Melanconium melanoxanthum*, B. & Br.)

This species is especially abundant on the midrib and sheath of decaying palm fronds, more particularly *Cocos nucifera* and *Oncosperma fasciculatum*. In its usual damaged state it presents a patch of scattered black spores, mixed with thin, irregular yellow scales; and beneath this mass are circular cavities in the tissue of the host plant: it answers then to Berkeley and Broome's brief description, 'Pustulis orbicularibus erumpentibus floccis granulisve flavis cinctum: sporis subglobosis angulatisve' (Journ. Linn. Soc. 14 (1875), p. 89).

As the decaying palm fronds afford a favourable medium for the growth of many other fungi, it is difficult to obtain exact evidence concerning the early stages of this species, more especially as all the individual fructifications of its extensive colonies appear to mature at the same time. The earliest determinable stages are indicated by a slight elevation of the cuticle of the host plant: a section through one of these pustules shows that it consists of a spherical mass of fungus tissue occupying a cavity excavated in the epidermis and the underlying tissues, but not more than 150 μ deep. This mass is entirely pale yellow, and consists of fine hyphae approximately parallel to each other and perpendicular to the surface of the host (Pl. XXIV, Fig. 1). Before the pustule bursts, spore formation commences in the centre of the sphere, so that a vertical section through an older pustule shows a blackish central circle of spores completely surrounded by a narrow yellow zone of hyphae. When the pustule opens, the ruptured cuticle forms a ring the height of which depends to a great extent on the nature of the host plant. On palms whose epidermis decays fairly rapidly, the open pustule is practically level with the surface of the frond, but in *Oncosperma* which has a tough, polished, persistent cuticle, the latter forms a ring which causes the open pustule to be raised about 100 μ. This is an unimportant detail, but it has to be insisted upon, because it is on just such details that species have been established from gatherings of tropical fungi. If the cuticle is light coloured, the open pustule is surrounded by a blackened area, but this is not noticeable on *Oncosperma* in which the cuticle is dark red-brown.

The open pustule (Figs. 2, 3) is circular, or sometimes oval, ·4–1 mm. diam., raised in some cases ·1 mm. above the surface. When it first opens, it forms a conical mound, apparently of yellow hyphae. The growth of the underlying central sporiferous hyphae pushes aside this cap, and leaves a black disk of spores surrounded by a rather irregular yellow ring. The hyphae of the unopened pustule which form the clear yellow sides and converge at the top do not bear spores; they are cemented together by a yellow secretion, and consequently, when the pustule opens, the upper part of the yellow zone breaks up. The fragments take the form of irregular thin plates, each composed of a few layers of parallel hyphae (Fig. 13). The upper part of the yellow ring which surrounds the open pustule consists chiefly of these fragments, and the ring appears granular under a low magnification. In some cases the sides remain intact and form an upright or recurved 'pseudoperidium', but this is always brittle and more or less covered with loose scales. The ring in the majority of specimens is an annulus of fragments; and similar fragments, dispersed by wind, rain, or insects, lie scattered round the open pustules. The breadth of the ring varies according to external conditions: as a rule, the loose scales are blown or washed away, and the yellow ring is very narrow, being only the free edge of the yellow hyphae in the substratum (Figs. 4, 5). In order to obtain undamaged specimens, they must be developed in the laboratory. This brittle yellow layer surrounding the sporophores is one of the chief characteristics of the genus.

In vertical section (Fig. 7), the fructification is cup-shaped, and sunk into the substratum for about 100–150 μ. There is no parenchymatous perithecial wall. When the pustule has opened, it consists entirely of erect close-set, parallel hyphae springing from the base of the cup. Those in the centre are free from each other and form the conidiophores, and are hidden by the black spores. The spores do not extend right to the base and therefore the column changes to clear yellow below. The hyphae towards the periphery are nonsporiferous, and thus the black central column is surrounded (in section) by a clear pale yellow zone of varying width, which appears at the surface as a yellow narrow ring or wall round the black disk, after the loose scales previously mentioned have disappeared.

In old specimens, the outer layer of the yellow hyphae, i. e. that which is in contact with decaying palm tissue, turns black, but the hyphae are parallel, not interwoven. When this has happened, the pustule in vertical section appears cut off from the decaying host tissue by a black cup, unless the section is almost exactly median: the median section shows that the black cup is not continuous, but that the yellow hyphae pass into the tissues of the substratum through a central aperture about one-third the diameter of the pustule. All the hyphae are approximately vertical and spring from the central aperture at the base of the cup.

The spores (Fig. 12) are subglobose or irregularly oval, or polygonal

with rounded angles, at first yellow, then fuscous, and finally black. They measure 14–19 × 12–14 μ, and are flattened, about 6–7 μ thick. Immature spores have a large central gutta. They are borne alternately on the erect hyphae on short pedicels attached to the middle of one of the flat sides, like the peridiola of *Cyathus* (Fig. 11). The oldest spores are at the top: the sporiferous hyphae appear to grow continuously and produce new spores below. These hyphae are about 2 μ diameter, sometimes slightly roughened, and are free from one another: the exterior yellow hyphae are 2–3 μ diameter, and are bound together by a yellow secretion.

In this stage it appears to be *Phaeodiscula gonospora*, Penz. & Sacc., with its subspecies *atrata*, *atratula*, and *minutella*. The differences between these subspecies depend on the size of the spores (which will be discussed later) and the varying appearance of the yellow ring surrounding the pustule: the latter feature depends almost entirely on weather conditions, the age of the specimen, and the nature of the host plant. *Melanconium profundum*, Penz. & Sacc., is a closely allied species, if indeed it is not the same: and the same may be said of *Melanconium Yatay*, Speg. *Graphiola macrospora*, Penz. & Sacc., described by these authors in 'Diagnoses Fungorum novorum in Insula Java collectorum', but omitted from the later 'Icones Fungorum Javanicorum', appears to be identical with the present species.

But the form described above is not the complete fructification: it is only the 'fair-weather' form, which is found in fairly dry situations or when two or three days' rain is succeeded by dry weather. It is, in fact, a case of arrested growth. In wet weather, growth does not cease when the pustule bursts, but the fructification lengthens out into a yellow cylindrical column up to 2 mm. high. In many cases there is then no ring at the base except the ruptured cuticle of the host (Fig. 9), but in others the outer layers of the yellow zone cease growth, while the inner layers grow on to the top of the column: the column then has a basal ring of yellow fragments (Fig. 8). This difference appears to depend partly on the breadth of the yellow zone. The outer yellow wall is slightly roughened and striate, extremely fragile, and at the most about 25 μ thick. It consists of parallel hyphae cemented together, and is, in fact, a continuation of the ring of the 'fair weather' form. The interior of the column (Fig. 10) is simply a black mass of loose spores and fragments of conidiophores almost down to the base. The structure may be compared to a sack of coal. The fragile outer wall frequently splits longitudinally, as shown in the figure. The perfect form is exactly cylindric, but one often finds specimens constricted in the middle as though there had been a temporary cessation of growth. It is rather remarkable that one never finds immature spores in the free column: all the spores appear to be produced at the base and to be carried upwards by the growth of the sporophores, and, as the cylinders are always filled up to the brim with spores, the whole column must grow at the same rate. They break up

at the slightest touch, and, therefore, the usual gathering consists of irregularly broken columns surrounded by masses of black spores mixed with irregular yellow scales. The latter have exactly the same structure as the scales which are found covering the newly opened 'fair weather' form: the yellow conical mound in the latter case consists of fragments of the tissue which should have grown out as the wall of the column. Berkeley and Broome's specimen of *Melanconium melanoxanthum* consists chiefly of fragments of these columns.

The suggestion that we may be dealing with two species is easily met. The spores, sporiferous hyphae, yellow hyphae, and structure of the base are identical in the two forms, and, in addition, the columnar form can be grown from the 'fair weather' form. If a piece of palm frond bearing the latter is placed in a Petrie dish with just sufficient water to cover the bottom, the yellow columns grow out from the pustules in two or three days. It is evident from this that the hyphae are capable of continuous growth; and in this respect the fungus differs from most of the Melanconiaceae, in which the spores are distributed almost as soon as the pustule opens and are not succeeded by a fresh crop from the same conidiophores.

Another variation occurs in very wet weather, or can be induced by keeping the substratum too moist. I obtained it first by saturating with water a piece of palm frond bearing unopened pustules from which it was hoped to grow perfect examples. When the pustules burst there grew out from each a circular tuft of white hyphae to a diameter of about two millimetres. Apparently there had been a mistake in the identification of the pustules, or they had been attacked by some mould. However, vertical sections showed that the hyphae were not parasitic, but were continuous down to the base of the pustule: they were the same diameter as the normal hyphae of *Endocalyx*, and irregular yellow granules, sometimes adhering to a hypha and sometimes free, were mixed with them. After a few days, when the cultivation had become drier, the usual yellow column was produced in the centre of each white tuft. In this case the columns appeared at first sight to be attached to the substratum by a fibrillose disk.

The yellow colouring matter is soluble in absolute alcohol, chloroform, or ether, and to some extent in ammonia since the latter separates the individual hyphae of the yellow wall. It appears to form a continuous layer over the external hyphae and bind them together, but when the latter grow very rapidly (as in the instance cited above) it is left in irregular lumps, and the hyphae are white (in mass) and free from each other. The colour varies from bright yellow to greenish yellow.

The spores of *E. melanoxanthus* on *Cocos nucifera* measure 14–19 × 12–14 μ and are 6–7 μ thick: those of *E. melanoxanthus* on *Oncosperma fasciculatum* appear to be uniformly smaller, and measure 10–17 × 8–13 μ.

In all other respects these fungi are identical, and when the spores vary so much it seems scarcely wise to separate the second form even as a variety. In both cases the spores vary from angular to circular.

Phaeodiscula gonospora, Penz. & Sacc., is apparently the fair-weather form with a yellow rim and spores 9–10 × 8–9 μ, angular and compressed; subspecies *atrata* has a black rim, and spores 9–10 μ diam., less angular and more compressed; subspecies *atratula* has a black rim, and spores 12–15 μ diam., more distinctly angular; and subspecies *minutella* has a yellow rim, and spores 10–12 μ diam. All these spore measurements fall within the limits obtained from a single pustule, and the yellow or apparently black rim is only a question of age or weather.

The variations of *E. melanoxanthus* afford a good example of the opportunities of describing species which a single tropical fungus offers to those who have not watched its growth.

ENDOCALYX CINCTUS, n. sp.

This species occurs on decaying fronds of *Oncosperma fasciculatum*, but is rather rare. The only specimens at present available are fullgrown. It differs in several important particulars from *E. melanoxanthus* and is more closely allied to *E. Thwaitesii*.

The mature fungus (Figs. 14, 15) is about 1·5 mm. high, and consists of a narrow cylindrical stalk expanding into a yellow, more or less funnel-shaped structure at the top. Viewed externally, the stalk appears to consist of two distinct parts. The lower part is black, rough, and cylindric, ·5–·6 mm. high, about ·1 mm. in diameter, expanding somewhat abruptly at the top to sometimes twice the diameter, and terminating with a horizontal upper edge. From the centre of the flattened top springs a narrower column, ·06–·1 mm. diameter, blackish brown at the base, becoming yellow upwards: this part is either of uniform diameter for the greater part of its length and then expands suddenly at the apex, or is regularly funnel-shaped throughout its whole length. The upper part for a depth of about ·5 mm. splits longitudinally into five or six spreading lobes, rectangular in outline and usually recurved. This division concerns the outer wall of the column only: the inner portion is a mass of black spores. The spreading lobes of the outer wall are very brittle and break off in plate-like fragments, up to ·5 mm. long. The top of the funnel is ·25–·5 mm. in diameter.

In vertical section (Fig. 16) the lower black part of the stalk is seen to be a hollow cylinder of vertical, more or less parallel hyphae. The hyphae on the inner surface, which are only slightly blackened, are about 2 μ diameter, but the remainder are converted into a black carbonaceous mass in which individual hyphae can scarcely be recognized. If the fungus is soaked in ammonia, and then pressed under a cover glass, the black

cylinder splits longitudinally into four equal strips: from this and the arrangement of the inner hyphae, it is probable that all the carbonized hyphae were vertical and parallel.

This cylinder surrounds and is adherent to an inner stalk of parallel, vertical, hyaline or yellowish hyphae, 1·5–2 μ diam., bound together into a solid mass, but separating on soaking in ammonia. This inner stalk emerges from the flattened top of the black cylinder and the hyphae remain united for a short distance. The inner hyphae are then yellow, while the outer are slightly brown in mounted specimens. The inner then separate and produce the conidia, while the outer remain united and form a thin, yellow, brittle wall, which splits longitudinally at the top.

The outer wall is identical in structure with that of *E. melanoxanthus*. The present species differs from *melanoxanthus* in the possession of a distinct yellow stilbum-like stalk, surrounded by a close-fitting carbonaceous cylinder at the base. The yellow stalk corresponds exactly in structure with the base of the pustule of *melanoxanthus*, and may be regarded as a prolongation of the latter; while the black cylinder is homologous with the black hyphae which almost enclose the sunken base in mature specimens of *melanoxanthus*.

Nothing is yet known about the early stages of this species. The black column may possibly arise from the subepidermal pustule after the manner of a perithecium, and then open to produce the central, yellow, stalked funnel; or it may be that all the hyphae grow at the same time from the pustule and become differentiated in the course of elongation. As the black column has not the structure of a perithecium, and shows no broken edge at the top, it is probable that the latter view is correct.

One rather important difference between this species and *E. melanoxanthus* arises in the relation of the fungus to the substratum. The hyphae which form the fructification of the latter spring from a narrow base, and expand to form the column, while in *E. cinctus* the hyphae converge towards the foot of the column in the tissues of the substratum.

The crowded conidiophores are simple, and bear spores on short alternate lateral pedicels as in *E. melanoxanthus*, the spore being attached in the centre of one of the flat sides. The pedicels are fairly close together and the hyphae are extremely fine, so that, in section, the spores appear to be arranged in more or less vertical rows. The individual sporiferous hyphae have a somewhat zigzag course. The spores (Fig. 17) are at first yellowish, and finally black, circular or ovoid, compressed, and thick-walled, 9–11 × 11–12 μ: immature spores are covered with minute scattered warts, but these cannot be detected on the opaque ripe spores.

ENDOCALYX THWAITESII, B. & Br.

This species was described twice, (1) as *E. Thwaitesii*, 'Ore insigniter laciniato, laciniis elongatis; stipite gracili, elongato. On dead sticks. Spores varying from globose to oval, 15–20 μ,' and (2) as *E. psilostoma*, B. & Br., 'Ore primum integro, dein fisso; stipite brevi crassiore. With the last. Spores 20–25 μ.' Berkeley and Broome give a somewhat imaginary figure of the first species (Fig. 18), showing a sessile basal cup, five times the diameter of the stalk, with an incurved upper edge and apparently floccose: from the centre of this cup springs the cylindrical stalk which expands like a calyx at the top: the upper edge of one specimen is divided into about nine fairly long triangular teeth: the other is more divided and the teeth split up into long thin fascicles of hyphae. The stalk and 'calyx' are rough with minute recurved scales.

The herbarium specimens are both on *Oncosperma*. They were apparently gathered by Thwaites at the same time, and form one of his numbers (1048). Berkeley and Broome give 'e basi crassa oriundum' as one of the generic characters, but *E. psilostoma* has no such base, and it is not a normal part of the fungus. It is this thick base which the artist has represented as a cup: it forms a solid annulus round the stalk of *E. Thwaitesii*, and consists of a few hyphae and fallen spores mixed with the coarse brown epidermal hairs of the outer surface of *Oncosperma*. It is not formed in the same way as the basal ring of *E. melanoxanthus*.

The structure of the fungus is best determined from the less damaged specimens which constitute *E. psilostoma*. These have a stilbum-like stalk about ·1 mm. diameter, blackish at the base, and becoming yellow upwards. They expand slightly at the top, forming a small funnel, but the recurved, yellow, spreading lobes, such as are present in *E. cinctus*, have been broken off, and only the base of the funnel is left. Specimens in this condition no doubt authorized Berkeley and Broome's statement, 'Ore primum integro.' The size and colour of the hyphae, the internal structure, and the arrangement of the spores are identical with those of *E. cinctus*, but the spores (Fig. 19) are more strongly warted or spinulose, regularly oval or circular, less compressed than in *E. cinctus* or *melanoxanthus*, blackish brown, 17–21 × 16–19 μ. It only differs from *E. cinctus* in the larger warted spores, and in the absence of a black basal cylinder enclosing the stalk. Its total height is about 1·25 mm. Under a high magnification the warted edge of the spore appears yellow.

The specimens of *E. Thwaitesii* are weathered and brown, and in nearly half of them only the stalk is left. The base of the stalk is surrounded by the upturned cuticle of the substratum, or by the annulus which Berkeley and Broome describe as a solid base. Where this annulus is present, the stalk passes through it loosely and is not united to it in any

way. The 'calyx' at the top of the stalk remains in fourteen cases; in four of these it is composed of oblong lobes as in *E. cinctus*, and in the other ten it splits up into a brush of fascicles of hyphae. Berkeley and Broome's two figures show both these forms, though the lobes are too pointed in the first. The specimens are only slightly rough, not scaly as in the figures.

The conidia and conidiophores are the same as those of *E. psilostoma*, and it is undoubtedly the same species.

But the greatest difference between this species and *E. cinctus* and *melanoxanthus* lies in the structure of the lobes of the cup or funnel: in the latter they are brittle and rectangular, but in most of the specimens of *E. Thwaitesii* they divide into fine strands of hyphae and are flaccid. Such specimens, therefore, lack one of the chief characters of the genus, if it is to include the other species. The yellow substance which should cement the external hyphae into a continuous sheet is scattered in irregular granules (up to 4 μ diam.), so that the narrow strands appear beaded: and the conidiferous hyphae are roughened in the same way. The strands of hyphae are therefore flexible. The comparison with the other two species to which it is undoubtedly related suggests that this condition is abnormal, and this view is supported by the occurrence in the same gathering of specimens in which the hyphae are united into plates. It has been shown that when *E. melanoxanthus* is supplied with a large quantity of water, it produces first of all a white tuft of hyphae with irregular yellow granules scattered through it. This appears to afford a clue to the abnormalities of *E. Thwaitesii*. It seems probable that the specimens developed in very wet weather, and that the yellow binding material has therefore been excreted in irregular granules instead of in a continuous sheet. Similarly, the annulus at the base might be formed by a tuft of hyphae such as occurs under the same conditions in *E. melanoxanthus*.

Berkeley and Broome's type specimens are undoubtedly in a damaged condition, and the above seems the most probable explanation of their present state; but, until fresh specimens have been collected, we can only be certain that it is a stalked form, and differs from the other two in its coarsely-warted or spinulose spores. When the specimens of *E. cinctus* were first gathered, it was thought that this might be identical with *E. Thwaitesii*, but as no trace of a black basal cylinder can be detected in the herbarium specimens of the latter, this view had to be abandoned.

Classification.

Berkeley and Broome placed the genus *Endocalyx* in the Myxomycetes, a position which its hyphal structure at once negatives. Penzig and Saccardo named the 'fair-weather' form of *E. melanoxanthus*, *Phaeodiscula*, which would include it in the Sphaeropsidales, but the absence of a distinct

perithecium contradicts this view and indicates that it must stand either in the Melanconiales (where Berkeley and Broome placed *E. melanoxanthus*), or in the Hyphomycetes.

In its general appearance and habitat, the commonest species, *E. melanoxanthus*, suggests a reference to *Graphiola*, but there is nothing in the structure to support this view. According to Ed. Fischer (Bot. Ztg., 1883), *Graphiola phoenicis*, Poit., is composed of upright parallel hyphae: the outer are united into a thick black peridium, while the inner grow out as a yellow column, but this column is not enclosed by a sheath as in *Endocalyx*. 'Eine Vermuthung, die bei nur makroskopischer Betrachtung leicht aufkommen kann, ist die, dass das aus dem Fruchtkörper hervorragende säulenförmige Gebilde, da es eine einheitliche Oberfläche zeigt, von einer Membran umschlossen sein müsse. Jedoch habe ich bei genauerer Untersuchung hier niemals eine Hülle oder dergleichen wahrnehmen können, immer waren nur Sporen und Hyphenbündel da' (Fischer, loc. cit., p. 763). Moreover, there are no sterile hyphal bundles among the conidiophores of *Endocalyx*, and the arrangement of the spores differs completely from that of *Graphiola phoenicis*. In all essential details, *Endocalyx* bears not the slightest resemblance to *Graphiola*.

It seems, however, probable that some of the species which Fischer accepted as *Graphiola*, e. g. *Graphiola congesta*, Berk. & Rav., *Graphiola* (?) *disticha* (Ehrenb.) Lév., *Graphiola* (?) *compressa*, Fischer, might be more closely allied to *Endocalyx*. In all cases the specimens were scanty or imperfect. *G. congesta* differs from the type in having a *strongly developed yellow inner peridium*, and apparently *no sterile hyphal bundles*, but Fischer decided that the arrangement of the spores agreed with it as far as the material allowed him to judge. The type specimens of *G. disticha* resembled *Graphiola* externally, but the peridia contained 'eine Menge aneinandergelegter dreiseitig prismatischer Säulen, bestehend aus ganz niedrigen Gliedern, die leicht auseinander gehen'. This is so contrary to Léveille's description that Fischer suggests that another fungus had developed in the perithecium. Evidently he had not then had the enlightening experience of comparing fresh specimens of tropical fungi with their so-called descriptions. Fischer bestows the name *Graphiola compressa* on a specimen which he admits is quite undeterminable: it is to be regretted that such specimens are not discarded. Under the circumstances, the question of the identity of these 'Graphiolas' with any other genus must remain undecided, but *G. macrospora*, Penz. & Sacc., appears from the description to belong to *Endocalyx*. The describers of the last-named write 'Exemplaria non perfecta, hinc stirps adhuc dubia et ulterius inquirenda': and it differs from *Graphiola* in its dark spores.

Except for the peculiar yellow sheath, there seems nothing to prevent the inclusion of *Endocalyx* among the Hyphomycetes. In this group,

Microcera has a loose sheath which is longer than the ripe sporodochium, and *Fusarium* has an adherent sheath which envelopes the head of conidia, but in both these cases the sheath is not brittle and the hyphae composing it are joined by ladder-like connexions instead of being merely cemented together. The parallel therefore is not exact. The stalks of *E. cinctus* and *E. Thwaitesii* suggest an alliance with *Stilbum*, though the black sheath of the first-named is a departure from the usual structure in that genus. On the whole *Endocalyx* would appear to stand best in the section Stilbaceae-Phaeostilbeae, though the arrangement of the spores seems to have no parallel in that section.

Riccoa, Cav., appears at first sight to resemble *Endocalyx*: the stalk expands at the top into a cup in which the sporophores are produced, and the spores are borne along the sides of the latter. But the stalk and cup are parenchymatous, and the sporophores are rather widely separated and compound, i.e. formed by the fusion of several hyphae: the lateral arrangement of the spores, as far as can be understood from the description and figures, is due to a progressive separation of these hyphae upwards, so that the spores are really terminal on the individual hyphae. As in *Graphiola*, the spores are hyaline.

ENDOCALYX, B. & Br. (amended).

Synnemata (?) stipitata vel sessilia, basi innata, ex hyphis parallelis verticalibus composita: hyphae internae supra disiunctae, conidiferae: hyphae externae in membrana fragili, cylindrica vel infundibuliformi conglutinatae: conidiophora simplicia, libera, densissime conferta, deorsum coalescentia: conidia pleurogena alternata, stipitellata, continua, fusca, compressa.

ENDOCALYX MELANOXANTHUS (B. & Br.) *Melanconium melanoxanthum*, B. & Br. Journ. Linn. Soc. 14 (1875), p. 89. Synnemata ·4–1 mm. diam., vel immersa, orbicularia, laminis flavis et margine flavo cincta, vel exserta ad 2 mm. alt., flava, cylindrica, saepe fissa: hyphae externae, 2–3 μ diam., membrana fragili, cylindrica, flava ad 25 μ crassa conglutinatae: hyphae internae, ad 2 μ diam., conidiferae, in matrice solum conglutinatae: conidia subglobosa vel angulata, primum flava, dein nigra, compressa, levia, continua, 14–19 × 12–14 μ, 6–7 μ crassa. In frondibus palmarum emortuis, Ceylon.

ENDOCALYX CINCTUS, n. sp. Synnemata, 1·5 mm. alt., erecta, cylindracea, supra infundibuliformia, flava, ex hyphis parallelis, 1·5–2 μ crassis, conglutinatis, formata: deorsum vagina nigra, cylindrica, ad ·6 mm. alt., ·1 mm. diam., ex hyphis parallelis composita, cincta: hyphae externae supra in membrana fragili, flava, striata, dentibus quadratis recurvis fissa, conglutinae: hyphae internae deorsum in stipite conglutinatae, supra libera, confertae, conidiferae: conidia globosa vel ovata, compressa, primum flava

et minute verrucosa, dein nigra, 9–11 × 11–12 μ. In frondibus palmarum emortuis, Peradeniya, Ceylon.

ENDOCALYX THWAITESII, B. & Br. *E. psilostoma*, B. & Br. (loc. cit.). Synnemata erecta, circa 1·5 mm. alt., ·1 mm. diam., cylindrica, supra calyciformia, flava, ex hyphis parallelis, 1·5–2 μ crassis, composita: [hyphae externae supra in membrana fragili, flava, dentibus recurvis fissa, conglutinatae]; hyphae internae supra liberae, conidiferae, confertae: conidia, ovata vel globosa, minime compressa, atrofusca, verrucosa vel echinulata, 17–21 × 16–19 μ. In frondibus Oncospermatis emortuis, Peradeniya, Ceylon.

EXPLANATIONS OF FIGURES IN PLATE XXIV.

Illustrating Mr. Petch's Paper on the genus *Endocalyx*.

Endocalyx melanoxanthus (B. & Br.)

Fig. 1. An unopened pustule of yellow hyphae. × 20.

Fig. 2. Open pustule, viewed from above, showing the yellow ring and black disk of spores. × 20.

Fig. 3. A similar pustule viewed laterally, raised by the upturned cuticle of the substratum. × 20.

Fig. 4. An old weathered pustule: the loose scales of the ring blown or washed away. × 20.

Fig. 5. The same, viewed laterally. × 20.

Fig. 6. A recently opened pustule, with a very irregular granular ring. × 40.

Fig. 7. Vertical section through an open pustule: column of sporophores and spores in the centre, surrounded by a zone of agglutinated yellow hyphae, which appears as a ring at the surface: the outer layer of hyphae in contact with the substratum is blackened. × 40.

Fig. 8. The perfect columnar form, surrounded at the base by the upturned cuticle and a ring of yellow fragments. × 20.

Fig. 9. The columnar form without a basal ring: this shows a constriction which probably indicates a temporary cessation of growth: the lower part of the cylinder is split longitudinally. × 20.

Fig. 10. Vertical section through a fructification of the type of Fig. 8, showing the basal yellow zone, only the inner layers of which are produced to form the wall of the column. × 40.

Fig. 11. Part of a sporophore, with immature spores. × 300.

Fig. 12. Spores. × 300.

Fig. 13. A fragment of the yellow wall. × 300.

Endocalyx cinctus, Petch.

Fig. 14. Fructification with abruptly expanding funnel. × 20.

Fig. 15. Fructification with gradually expanding funnel. × 20.

Fig. 16. Longitudinal section of fructification; the conidiophores and external hyphae of the funnel are continued through the black cylinder into the substratum. × 40.

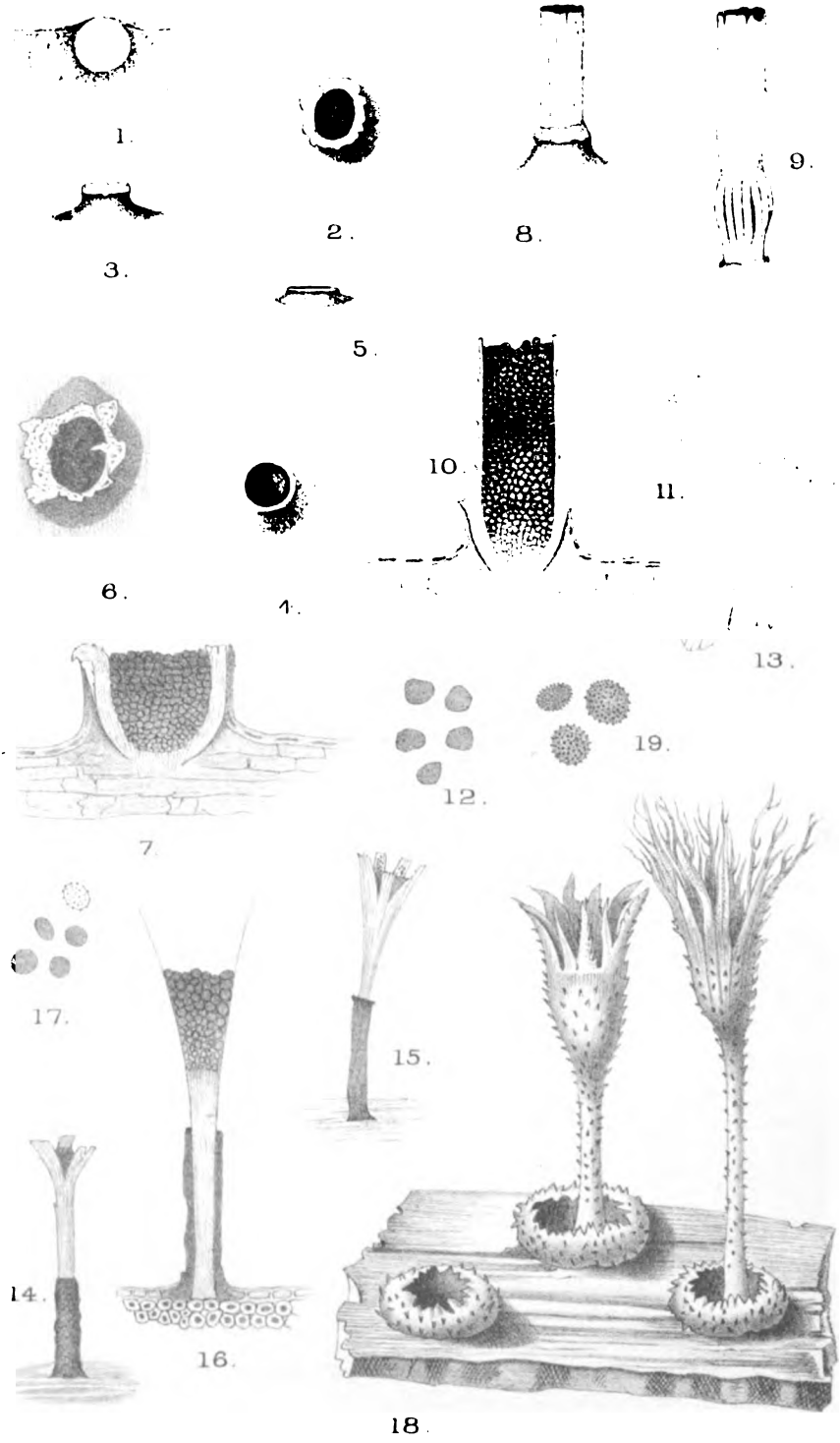
Fig. 17. Spores. × 300. The single hyaline immature spore is × 600.

Endocalyx Thwaitesii, B. & Br.

Fig. 18. Copy of Berkeley and Broome's figure. The substratum is *Oncosperma*, and should be fluted and tomentose. The cup at the base ought to be an annulus, through which the stalk passes loosely. The whole fungus should be minutely roughened (? normal), not floccose as in the figure. × about 50.

Fig. 19. Spores. × 300.

N.B.—In Figs. 7, 10, 16, the spores are represented diagrammatically as far as their size is concerned.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On Cavity Parenchyma and Tyloses in Ferns.

BY

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With Plate XXV and seven Figures in the Text.

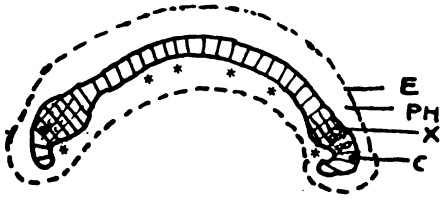
THE term 'cavity parenchyma' has been used to indicate a special kind of tissue occurring in the petioles of some Ferns in contact with the protoxylem groups. It consists of soft cells of a more or less irregular shape, forming vertical strands of loose large-celled parenchyma which replaces to a greater or less degree the first-formed elements of the xylem. Such parenchyma is formed from the one-layered sheath of soft cells immediately surrounding the protoxylem. The sheath cells in contact with the spiral vessels become enlarged and send ingrowths between the spiral thickenings into the lumina of the vessels. The result of further protrusion of the parenchyma cells into the vessels is that more or less spherical, swollen processes are formed, and the vessels become gradually broken up by the increasing pressure of these processes within the passages, the place of the spiral vessels being eventually taken by the parenchyma so formed. Russow proposed the name 'Lückenparenchym' for the soft cells filling up the cavity left by the tearing of the tissues near the protoxylem in *Marsilia*.

One of the earliest writers to mention strands of soft cells was Dippel ('64), who noticed them in *Osmunda* and in *Cyathea microlepis*; Terletzski ('84) also found them in *Pteris aquilina* and *Struthiopteris germanica*: he considered them to be part of the wood tissue, for, he says, they border directly on the protoxylem, and never directly on the bast cells and sieve-tubes, from which they are always separated by the parenchymatous sheath. Boodle ('01) gives a short account of cavity parenchyma in the Schizaeaceae; he states that in *Aneimia* there are three groups belonging to the conjunctive parenchyma adjoining the xylem, one at the median point, the others at a short distance from each hook; in *Mohria* there are also three groups of parenchyma (p. 359). In the Gleicheniaceae he mentions it as occurring in nearly all species, the cells frequently becoming thick-walled and lignified. Seward and Ford ('03), writing of *Todea*, state that 'the parenchymatous tissue abutting on the protoxylem strands occasionally forms small irregular cavities (cavity parenchyma),' p. 247; Seward ('99) mentions it also in

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

Matonia, p. 183, but no details are given. Farmer in the same year ('99), referring to *Helminthostachys*, writes that the protoxylem groups 'are often seen to border on a band of peculiar parenchyma similar to that which Russow termed Lückenparenchym. The cells of this tissue are large and thin-walled . . . the general impression which the observer acquires is that he is dealing with some kind of gland'.—p. 439. A somewhat unusual case of cavity parenchyma occurs in *Loxsoma*, where the cells become pitted and lignified, as recorded by Gwynne-Vaughan ('03), who states its formation to be due to a kind of tylosis. His figure showing some of the cavity parenchyma cells is reproduced in Pl. XXV, Fig. 17. Thus in all the large orders of modern *Filicales* and in *Marsilia* cavity parenchyma has been mentioned as present by different authors, and in one or two cases has been described, notably in *Loxsoma* by Gwynne-Vaughan, and in *Gleichenia* and *Trichomanes Prieurii* by Boodle. In the following account of cavity parenchyma I shall refer first to a number of ferns, giving details in each case, and shall then consider the function of the tissue.

A typical case of cavity parenchyma occurs in *Microlepia platyphylla*, one of the Polypodiaceae. In the petiole of this fern there is a single curved bundle, the ends being slightly hooked inwards; there is a group of protoxylem near each hook, and three or four groups lying between them (Text-fig. 1). The position of the parenchyma strands is shown by crosses.



TEXT-FIG. 1. Diagram of the petiolar bundle of *Microlepia platyphylla*.
E, endodermis. X, xylem.
PH, phloem. C, wood-parenchyma.

An enlarged view of one of the median strands in transverse section is shown in Fig. 1 of the plate. The cavity parenchyma is well developed, though it does not attain the great development of the corresponding tissue in the Tree Ferns, which are exceptional in this respect. The following description of the method of formation of cavity parenchyma can be applied with slight modifications to all Ferns. The tissue is most apparent in the thicker parts of the petiole near the base; it is continued up the rachis to the younger parts, gradually becoming less apparent towards the apex. Towards the end of the rachis the first stages of the development of the cavity parenchyma can be best seen. The xylem-sheath cells in contact with the protoxylem are seen to be somewhat enlarged: this enlargement results in their being pressed into close contact with the spiral vessels of the protoxylem; with further enlargement the parenchyma cells begin to press in between the spirally thickened portions of the vessels. The result is that a single parenchyma cell of the xylem-sheath generally shows several outgrowths, the constrictions representing

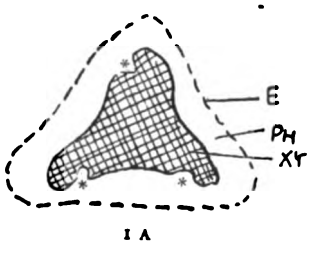
the points of resistance of the lignified parts of the vessel. The continued expansion of the parenchymatous processes within the lumina of the vessels results in the breaking up of the vessels, which thus early become non-functional. Frequently nuclei are distinctly seen in the bladder-like swellings in the vessels; the swellings are finally cut off from the original cell by a cross-partition. After breaking up one spiral vessel the parenchyma continues its growth, forming new processes which penetrate adjoining vessels, and these in turn become broken up. In *Microlepia* most of the spiral vessels become broken up, but in some Ferns the parenchyma destroys only a few of the first-formed vessels. Their place is thus taken by irregular parenchyma cells, frequently with large intercellular spaces between them, which form strands running the length of the petiole. Very frequently remains of the disintegrated vessels are seen in the spaces between the cells of the cavity parenchyma as small pieces of lignified thickening, sometimes two or three connected turns of a spiral, but generally small fragments considerably crushed. Such remains are to be noticed in the part of the parenchyma most recently formed; they are nearly always to be seen in the deep constrictions of the cells which have not ceased to expand, and they frequently give the cell itself a superficial appearance of being lignified at these points; in reality the tissue in this plant remains unlignified, though in various other Ferns lignification of the cavity-parenchyma cells does occur. In Fig. 1 three cavity-parenchyma cells are seen in cross-section at *cp*; they have replaced the protoxylem and have the appearance of extending a considerable distance into the phloem *ph*. The tissue is about one-fifth the width of the whole bundle. The metaxylem, which consists of a single row of tracheids, is seen at *xy*.

Cavity parenchyma has been recorded by Gwynne-Vaughan ('03) in *Davallia*, another member of the same sub-order. I examined *D. Griffithiana*, which has an elongated bundle, the xylem being surrounded by phloem about three cells deep; a group of cavity-parenchyma cells was seen at each end of the bundle, but the tissue, the cells of which are lignified in this species, did not occupy so large a space as in *Microlepia*. In *Nephrolepis* the vascular bundle is somewhat T-shaped or triangular, and the cavity parenchyma appears in the positions marked by stars in the Text-fig. 1 A, p. 404, opposite the three protoxylem groups. In longitudinal sections *Nephrolepis* shows very clearly the tylose-like swellings which occur in the spiral vessels. The Text-figs. 2, 3, 4, 5, show different stages of the ingrowth of the parenchyma.

Text-fig. 2 shows three cells of the xylem sheath which have formed small swellings in a vessel. Each cell had several swellings. Fig. 3 shows a stage in which the swellings have elongated, and have almost closed up the lumen of the vessel; Fig. 4 shows one swelling which has itself formed a swelling without being cut off from the original xylem-sheath cell by

a cell-wall; Fig. 5 shows a series of three swellings in three distinct vessels, all formed from a single cell and not separated by cell-walls. This is quite common in *Nephrolepis* which shows perhaps better than any fern the tylose-like nature of the cavity parenchyma. The cells may eventually become cut off by cell-walls.

In *Struthiopteris pennsylvanica*, one of the Aspidieae, the petiolar bundle is much elongated and the cavity parenchyma is only slightly developed; it can be seen adjoining the two groups of protoxylem near the ends of the bundle; at these points the sheath cells were slightly enlarged, and remains of the disintegrated vessels were to be seen between them. In longitudinal section some of the cells are seen to be crenulated



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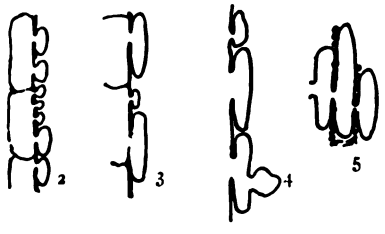
TEXT-FIG. I A. Diagram of petiolar bundle of *Nephrolepis*.
E, endodermis. XY, metaxylem.
PH, phloem.



TEXT-FIGS. 2, 3, 4, 5.
Stages of formation of cavity parenchyma in *Nephrolepis*.

in outline on the side adjoining the spiral vessels, but the development of the tissue is not striking.

In *Aspidium Filix-mas*, in which the bundles are numerous and more or less rounded in form when seen in transverse section, and the vascular elements are narrow, there was no trace of cavity parenchyma, but longitudinal sections showed a slight bulging of a few sheath cells, though no actual ingrowth to the vessels was to be seen. In the petiole of *Sadleria cyatheoides* a Fern of tree-like habit, which I examined as dried material only, the cavity parenchyma was slightly developed. In *Lomaria gibba*, a form having small round bundles, the parenchyma was distinct in some bundles and not to be seen in others in the same cross-section. The cells were about the width of one of the wide tracheids of the bundles. There was no cavity parenchyma in *Asplenium Ruta-muraria*, but one or two cells were to be seen which had apparently enlarged, and slightly crushed an adjoining spiral vessel. In the sub-order Pterideae I examined *Pteris aquilina*, *Gymnogramme ochracea*, and *Cheilanthes pulverescens*, in the first of which cavity parenchyma was recorded by Terletzski ('84), as mentioned above. It is also present in the two last-mentioned Ferns. In *Pteris* the tissue is more striking than in *Gymnogramme*;

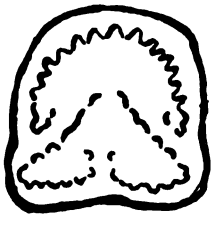
it attains a width about one-fourth or one-fifth that of the bundle. Fig. 2 is a longitudinal section of *Pteris aquilina* showing the general appearance of a strand of cavity parenchyma *cp*; it will be noticed that the strand is one or two cells wide, and that the cells themselves are very irregular in shape. Fig. 3 shows the expansion *ty* of the parenchyma cells in a spiral vessel. Disintegrated portions of the vessel are seen at px^1 and a nucleus at *n*; a pitted tracheid occurs at *mx*. Fig. 4 shows a more advanced stage in the cavity-parenchyma formation. Fig. 5 shows a transverse section of a single strand of cavity parenchyma. Johnson ('08) gives an account of some tyloses which he found in a preparation of the rhizome of the Bracken Fern; the appearance of the preparation, of which a photograph is given, exactly resembles the longitudinal sections of *Pteris* petiole showing cavity parenchyma. The soft cells figured appear to be in contact with the narrow elements of the xylem, the wider tracheid being at a greater distance. The preparation was made from a small detached piece of material by one of the students of his class, and it seems possible that either it may have been made from a piece of petiole, the tylose-like cells being cavity parenchyma, or that if cut from the rhizome it represents an unusual case of continuation of the cavity parenchyma in the rhizome. True tyloses formed in the wide pitted tracheids of stem-bundles are not known in recent ferns. In *Gymnogramme* and *Cheilanthes* there is a single petiolar bundle slightly hooked at each end, and in both these genera there is a strand of cavity parenchyma on the concave side of each of the hooks. *Gymnogramme* showed swellings of the xylem-sheath cells, one swelling sometimes giving rise to another without the formation of a dividing wall.

Although in a number of Polypodiaceous Ferns cavity parenchyma is well developed, it is in the histologically related group of the Cyatheaceae or Tree Ferns that the tissue attains its greatest development.

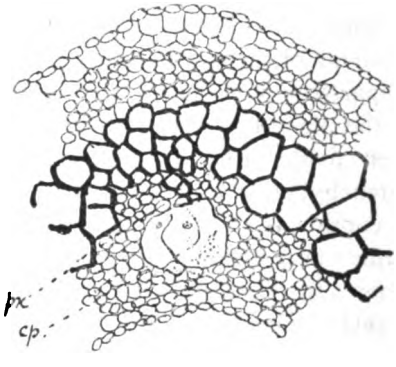
In *Alsophila excelsa* the vascular bundles of the petiole are elongated and crenulated, the protoxylem groups being situated in the little bays so formed. A single petiole has about forty or more groups and consequently has an equal number of cavity-parenchyma strands. A single strand attains a width of about one-third that of the bundle and the strands thus form a most conspicuous feature whether in transverse or longitudinal sections.

Text-fig. 6, p. 406, shows a transverse section of a petiole of *Alsophila excelsa*: in this section there were to be seen forty-four groups of protoxylem, each having a corresponding strand of cavity parenchyma. Longitudinal sections (see Fig. 6 in the plate) show that the cells are all quite parenchymatous, having thin cellulose walls, distinct nuclei and protoplasmic cell contents and that they are irregular in shape and have fairly large intercellular spaces. They replace many of the spiral elements of the xylem, and in transverse sections are seen to extend across the phloem, almost to the endodermis at times, as shown in Text-fig. 7, p. 406.

In *Dicksonia antarctica* the extent of a single strand is much the same as in *Alsophila*, though sometimes a little narrower; but the cells themselves are considerably smaller, as shown in Fig. 7 in the plate; and whereas in *Alsophila* a transverse section frequently shows only one very large cell representing the whole strand, in *Dicksonia* a similar section will show several smaller cells. Figs. 6 and 7 are drawn with a camera lucida to the same scale. In *Dicksonia* the cells are lignified and rather small, in *Alsophila* they are unlignified and large. In *Hemitelia*, which I examined only as dried material, the cells were arranged as in *Alsophila*, the strands in the two being almost exactly the same width and generally one cell wide. In *Cibotium princeps* (*Cyathea princeps*, Hook.) a strand is generally



TEXT-FIG. 6. Transverse section of petiole of *Alsophila excelsa* showing the form of the vascular bun



TEXT-FIG. 7. Transverse section of a single 'bay' in the vascular bundle of *A. excelsa*, showing cavity parenchyma *cp*: the protoxylem *px* is not entirely destroyed.

two or three cells wide and is about half the width of the bundle: the phloem immediately opposite the protoxylem is almost entirely displaced by the growth of the cavity parenchyma. Fig. 8 shows a transverse section of a portion of a bundle of *C. princeps*. In this species the tissue showed an even greater development than in the other Tree Ferns mentioned, and showed, moreover, an interesting point which I have noticed in no other Fern. Most of the cells of the cavity parenchyma are thin-walled and of the usual nature: others, on the contrary, are reticulately thickened and lignified, and have all the appearance of water-storing tracheids. They resemble the 'Speichertracheïden' described by Haberlandt ('96). In almost any transverse section, providing it was not too thin, these lignified cells could be seen. Some of them are very large, being about a quarter or a third the width of the bundle itself, thus forming a very prominent feature. Fig. 8 shows three of these tracheids and Fig. 9 shows an enlarged view of part of the same preparation; at *px* is seen the protoxylem, and at px^1 the spirally-thickened portions of some of the

disintegrated protoxylem vessels. In longitudinal sections these cells are seen to be of various shapes, generally having a more or less spherical form, and frequently having an elongated vertical portion attached. In Fig. 9 two of these portions are shown cut across at *et.* The tissue is formed in the usual way by the intrusion of the cells of the xylem sheath into the spiral vessels, which are thus disintegrated. In only one instance did I find parenchyma cells intruding into a wide pitted tracheid, and in this case the intrusive swellings were only small, and the markings on the tracheid-wall were for some reason unlignified. The other tracheids adjoining the unlignified one were normal in character; possibly the tracheid had become injured in some way, and so was weakened. The lignified cells of the cavity parenchyma are usually not actually in touch with the xylem of the vascular bundle, but may be isolated among a number of parenchyma cells. Fig. 10 shows a group of these thickened cells of the cavity parenchyma in longitudinal view: as a rule they do not occur quite so many together. The cavity parenchyma cells are not lignified until the tissue has developed to a considerable extent and the spiral vessels are broken up: they do not become lignified when they are protruding as bladder-like processes into the lumina of the vessels: such lignification would, of course, prevent the further expansion and growth of the cell. Miss Jordan ('03) found that in an herbaceous stem of *Cucumis sativus* there occurred tyloses having lignified walls, this lignification preventing the tyloses from extending far into the vessels, so that they never completely closed up the passages. The tyloses she figures resemble in appearance the lignified cells of the cavity parenchyma of *Cibotium*, which are formed in the first place by a process of tylosis, the difference being that whereas in *Cucumis* they are found actually within the lumen of the vessel, in *Cibotium* they have taken the place occupied by the spiral vessels of the protoxylem. The cells in *Cibotium* recall to a slight extent the water-storing cells or transfusion tissue found in certain fossil plants, for example, in *Megaloxylon*, and the centripetal wood occurring in the peduncles and petioles of recent Cycads.

In the Hymenophyllaceae cavity parenchyma has been mentioned by Boodle in *Trichomanes Prieurii*, while absent in other species. In *Hymenophyllum* I found that though there was no trace of tylose-like formations, the spiral elements were broken up and the parenchyma cells enlarged; the narrow woody elements seemed to be broken up by the pressure of the swollen parenchyma cells outside, not by the pressure of intrusions into the lumina of the vessels; such a distinction in the formation of cavity parenchyma is, of course, one of degree only. In the Gleicheniaceae and in the Osmundaceae in *Osmunda* and *Todea*, cavity parenchyma has been recorded, as mentioned above. In the last named, the intercellular spaces in the tissue are large; the protoxylem seems to

disintegrate, and its place may not be taken to any great extent by the xylem-sheath cells, so that frequently an actual cavity is seen.

In the Schizaeaceae Boodle recorded the tissue in *Aneimia Phyllitidis*. I examined *A. fraxinifolia*, which is regarded by Hooker as a subspecies of *A. Phyllitidis*; in this form there were four groups of cavity parenchyma in the petiolar bundle; the cells were lignified, and the nuclei very distinct. I found the tissue also in *A. tomentosa* but none in *Lygodium*.

In *Ophioglossum* I found no cavity parenchyma, though the protoxylem cells become somewhat crushed by the soft cells adjoining them, and in *Botrychium Lunaria* and *B. virginianum* the same is the case. In *Helminthostachys* cavity parenchyma was recorded by Farmer as noted above. Such parenchyma occurs both in the fertile and sterile leaf-stalks, but is more evident in the sterile stalks. There are generally four or five enlarged cells to be seen in connexion with the protoxylem elements in any transverse section. Longitudinal sections show that the cells apparently crush the spiral vessels by lateral pressure; I found no definite tylose-like growths, such as occur, for example, in *Pteris*, though the edges of some of the cells were slightly crenulated. Fig. 11 shows a semi-diagrammatic view of a cross section of *Helminthostachys* and Fig. 12 is a longitudinal section of a single vascular strand.

In the group of Marattiaceous Ferns Brebner ('02) has recorded and described cavity parenchyma in *Danaea*. He says: 'the cavity due to the breaking down of the protoxylem is filled with parenchyma owing to the increase in size, accompanied by a greater or less amount of division, of the adjacent living cells . . . It is probably not of any physiological importance, being simply a case of non-pathological hernia, so to speak' (p. 544). In *Marattia laxa* and *Angiopteris evecta* cavity parenchyma is well developed. In the former the tissue can be seen opposite most of the bundles, though not always opposite the very small ones. There may be one or more groups to each bundle, according to whether it is short or elongated. Generally the cells lie in a little 'bay' and occupy only the place formerly occupied by protoxylem, not extending into the phloem; they do not, in spite of the large dimensions of the plants, attain the great size of the same cells in the Tree Ferns; as a rule they are only a little wider than the phloem cells. In *Angiopteris* the tissue is more strongly developed than in *Marattia* and the cells may be three or four times the width of the ordinary xylem-sheath cells. The strand is about one cell wide. Fig. 13 shows a transverse section of a single bundle of *Angiopteris evecta* having one protoxylem group and a corresponding cavity-parenchyma strand *cp*: the nuclei are distinct.

In *Marsilia quadrifolia* among the Water Ferns the tissue is very well developed; it occurs most distinctly near the protoxylem strand at the apex of the triangular bundle, as shown in Figs. 14 and 15. At the other two

points it is not noticeable in transverse view, though in longitudinal view the cells are seen to be enlarged and crenulated. Fig. 16 is a section of the cavity parenchyma showing the lobed nature of the cells. In the Water Ferns, as in the Land Ferns, the cells grow out first into the lumina of the vessels by small swellings which enlarge within the passages.

With regard to the function of the cavity parenchyma thus widely distributed in Ferns, Dippel, writing of *Osmunda*, states that the strands are 'wahrscheinlich zur Aufnahme von Absonderungsprodukten bestimmt und den Harz-, Gummi und Milchsaftgängen der Phanerogamen an die Seite zu stellen'. Terletzski's view differed from this: his opinion was that the strands had 'keine andere Funktion als die Geleitzellen, da sie von diesen nur in Grösse und Form abweichen, aber im Bau der Wände und in Inhalt mit letzteren übereinstimmen' (Terletzski '84, p. 464). It is very natural at first sight to look upon the strands, as did Dippel, as special secretory passages, for their appearance is so very unlike the normal arrangement of the wood-parenchyma cells, but the view is incorrect. The cells are generally, it is true, of an ordinary parenchymatous nature, as Terletzski thought, but in a considerable number of Ferns, for example, in *Dicksonia antarctica* and in *Loxsoma* their walls are lignified. Such lignification might indicate that the function of the tissue was mechanical; the spiral vessels early become functionless, being broken up by the cells of the xylem sheath, and their place taken by a new tissue. Such a tissue would naturally be much stronger if the cells were lignified, and to a certain extent the tissue may have this function. In *Cibotium princeps* some of the cells are reticulately lignified, whilst others are entirely unlignified. Possibly this lignification is to compensate for the disintegration of the spiral vessels, the amount of conducting tissue being thereby very slightly lessened, but as the lignified cells are not all actually in contact with each other, and are often isolated, either singly or in groups, such a theory is hardly applicable. In the Equiseta, Strasburger states that the watery sap travels along the carinal canals and passes from one canal to another at the nodes by means of large pitted tracheids which form a connected mass. Large swollen parenchymatous cells near the tracheids frequently project into the carinal canals and have something the appearance of cavity parenchyma cells, though otherwise in the Equiseta the protoxylem is replaced by a canal which is used for water-conduction. But as in the Ferns, the ordinary tracheids composing the metaxylem are sufficient to carry the stream of water up the plant, it seems likely that the tracheids found in the cavity parenchyma of *Cibotium* are simply for storing water, and that in other Ferns where lignification of all the cells occurs, the tissue performs the same function. The need for such a storage of water in some Ferns is indicated in *Todea superba* in which Seward and Ford ('03) describe some tracheids of great breadth found here and there in the xylem

of the stem: 'the large diameter and short length suggest a storage rather than a conducting function,' p. 248.

At the same time the cells probably act to a certain extent as strengthening tissue. Thus in Phanerogams and Ferns there are instances of the lignification of tyloses resulting in cells which have all the appearance of water-storing cells.

Cavity parenchyma is to be regarded as a special tissue formed by the conjunctive parenchyma cells of the vascular bundles of the petiole, which replaces the first-formed elements of the wood, sometimes by simply crushing the spiral vessels, but generally by means of tylose-like swellings within the cavity of the vessels. True tyloses, that is, soft cells which enter the wide tracheids or vessels and remain within them, simply closing up the cavities by the formation of a pseudo-parenchyma, are unknown in recent vascular cryptogams, except in a case mentioned by Conwentz, who found tyloses in the old leaf-stalks of *Cyathea insignis*, and in the case mentioned by Johnson in the rhizome of *Pteris aquilina*; they occur frequently in monocotyledons and dicotyledons where, on account of the large amount of conducting tissue, the advantage of stopping up some of the wood vessels can be readily understood. In the fossil Fern petiole *Rachiopteris insignis*, now known to have belonged to *Zygopteris corrugata*, the xylem elements are often seen to be entirely closed up by soft parenchyma cells. It is a matter of some difficulty to explain the presence or function of these soft cells, on account of the fact that in the petiolar bundle the tracheids form a compact mass and are not separated from each other by any soft cells. Thus if the tyloses, generally so-called, are really formed by the ingrowth of the xylem-sheath cells, these cells must have been in a remarkably active condition to grow to such an extent as to fill all these wide tracheids. It is quite possible that such was actually the case, though one hesitates to ascribe to fossil Ferns an activity not present in recent Ferns, for even in the Tree Ferns, where cavity parenchyma attains its greatest development, and in *Nephrolepis*, where a single cell can be seen growing through two or three vessels, the activity of the sheath cells occurs only opposite the weak protoxylem vessels: the cells never enter the wide tracheids of the metaxylem. The formation of cavity parenchyma is, of course, of the same nature; but when one considers the usual extent of the tissue as compared with the extent of the whole bundle, it will at once be seen that there is a great difference between the activity necessary to form cavity parenchyma and that which would be required to form a pseudo-parenchyma throughout the tracheids of the bundle. Gwynne-Vaughan, in a paper read at the British Association at Leicester, 1907, showed that the middle lamella of recent fern tracheids consists of pectose which disappears as the plant becomes older, the lumina of the adjacent xylem elements being thus placed in communication with each other by the disappearance of the pit-

closing membrane. Such, he also showed, was the case in fossil Ferns, and as there would be no actual resisting membrane over the pits to break through, this renders it somewhat more likely that the cells of the xylem sheath could grow out from one tracheid to another, and so throughout the bundle, just as in the xylem-sheath cells grow out from one spiral vessel to another in the formation of cavity parenchyma. Another argument in favour of the theory that these soft cells in fossil Ferns may be due to the xylem-sheath cells is that a petiole of *Rachiopteris* (*Zygopteris*) *corrugata* was recorded by Weiss ('06) as showing two small lignified tyloses in one of the tracheids, the others being filled by cells having the usual unthickened walls. This, of course, indicates that the tyloses were formed by the ingrowth of a soft wood cell and not by a fungoid organism, for the latter would not be capable of such lignification. Such an example is a strong argument in favour of the view that the soft cells in the tracheids are all due to the ingrowth of soft wood cells. I cannot explain the presence of these lignified tyloses except as being due to an isolated soft cell enclosed within the xylem, such as one occasionally sees in ferns which have a compact mass of xylem. So far as I know, no reference has been made to specimens of *Rachiopteris* in which tyloses occur only in the tracheids at the periphery of the bundle, as one might reasonably expect if the soft tissue were formed from the xylem-sheath cells, for the tyloses in this case should be formed first in the tracheids immediately adjoining the sheath cells. I have seen slides of *Rachiopteris* in which some cells, especially the smaller cells, to the outside are empty and others are filled with 'tyloses': some of the tracheids in the interior of the bundle had the soft tissue, others had not: the arrangement was quite irregular. I incline to the opinion that the occurrence in some fossil Ferns of soft pseudo-parenchyma sometimes stopping up the entire mass of tracheids, sometimes stopping up only a proportion, is due to the presence of fungi which had entered the plant. The phenomenon of the expansion of the mycelium of a fungus in the cavities of wood vessels is well known. Referring to the mycelium of the Agaricineae ramifying in the wood of a host plant, Tubeuf and Smith ('97) write: 'The mycelium gradually spreads . . . into the vascular elements of the wood. . . . While previously it was simply filiform and furnished with numerous lateral hyphae, it now develops large bladder-like swellings, and at the same time the hyphae change into a kind of large-meshed parenchyma, which like the tyloses of many dicotyledonous trees completely fills up the lumina of the tracheids.' If the pseudo-parenchyma in fossil wood is due to the xylem sheath, one would expect to see in longitudinal sections evidences of the horizontal extension of these cells from one tracheid to another, such as one sees in modern Ferns, whilst if the tissue is due to fungi there would be less likelihood of noticing the passage horizontally of a fungal filament; the direction of extension of a fungal filament would be generally

vertical. In one longitudinal section of *Rachiopteris insignis* I noticed that in two wide scalariform tracheids adjoining each other, one was filled with pseudo-parenchyma, whilst the adjoining one contained none: there was not even a beginning of growth of the pseudo-parenchyma, as one might have expected, by analogy with cavity-parenchyma formation; in the latter case one vessel does not become entirely filled up with a tissue before sending out processes into the adjoining vessels. A long fungal filament ran vertically in the otherwise empty tracheid. On the whole, it seems to me that there is at least as strong evidence in support of the view that the pseudo-parenchyma in the tracheids of fossil Ferns is due to the presence of fungi as for the view that it is due to the xylem-sheath cells which fill up the lumina of the tracheids by a true process of tylosis.

To Professor Weiss, although I differ from him in my view as to the formation of soft cells in the tracheids of fossil Ferns, I wish to express my indebtedness for help in this piece of work and for the useful suggestions he has made to me from time to time.

UNIVERSITY OF MANCHESTER.
January, 1908.

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EXPLANATION OF PLATE XXV.

Illustrating Miss McNicol's Paper on Cavity Parenchyma and Tyloses.

Fig. 1. *Microlepia platyphylla.* Trans. sec. of part of petiolar bundle. *en*, endodermis *p*, pericycle; *ph*, phloem; *xy*, xylem; *cp*, cavity parenchyma.

Fig. 2. *Pteris aquilina.* Long. sec. of petiole showing cavity parenchyma.

Fig. 3. The same. Beginning of formation of cavity parenchyma by tylose-like intrusions *ty* into the spiral vessels *px*. *mx*, metaxylem; px^1, remains of spiral vessels; *n*, nucleus.

Fig. 4. The same. Later stage in the formation of cavity parenchyma.

Fig. 5. The same. Trans. sec. of petiole. *con. p.*, conjunctive parenchyma.

Fig. 6. *Alsophila excelsa.* Petiole, long. sec. showing cavity parenchyma.

Fig. 7. *Dicksonia antarctica.* Petiole, long. sec. showing cavity parenchyma.

Fig. 8. *Cibotium princeps.* Trans. sec. of a single 'bay' in the vascular bundle of the petiole. *xs*, xylem sheath; *t*, reticulately lignified tracheid.

Fig. 9. The same. Enlarged view of strand of *cp*. px^1, the remains of a disintegrated spiral vessel.

Fig. 10. The same. Long. sec. of a strand of cavity parenchyma. *et*, elongated tracheid.

Fig. 11. *Helminthostachys.* Semidiagrammatic view of trans. sec. of sterile leaf-stalk.

Fig. 12. The same. Long. sec. showing *cp*. *bp*, tracheids with bordered pits.

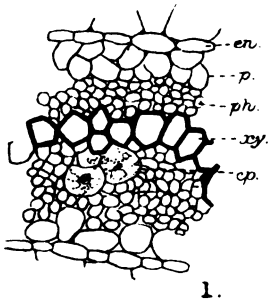
Fig. 13. *Angiopteris evecta.* A single petiolar bundle. *sc*, secretory cells.

Fig. 14. *Marsilia quadrifolia.* Bundle of petiole, trans. sec.

Fig. 15. *Marsilia quadrifolia.* Cavity parenchyma enlarged. *e*, cavity.

Fig. 16. *Marsilia quadrifolia.* Long. sec. of cavity parenchyma.

Fig. 17. *Loxsoma.* Cells of cavity parenchyma—after Gwynne-Vaughan.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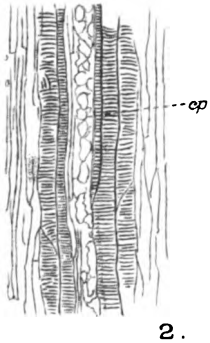
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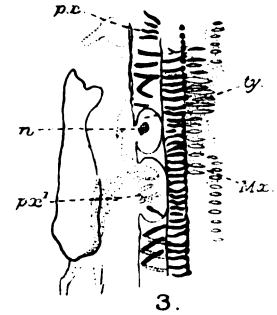
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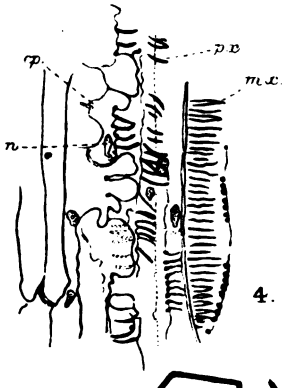
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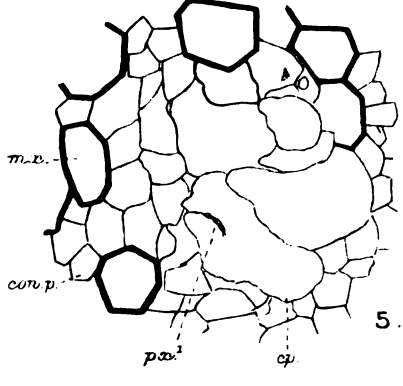
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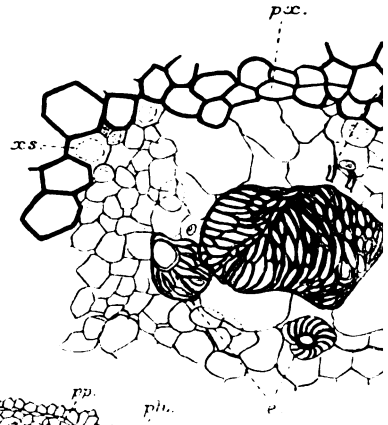
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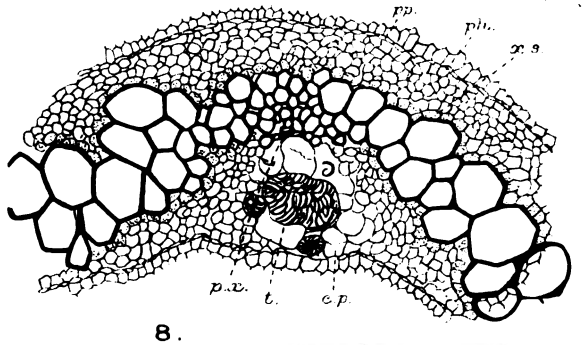
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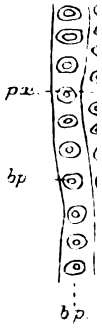
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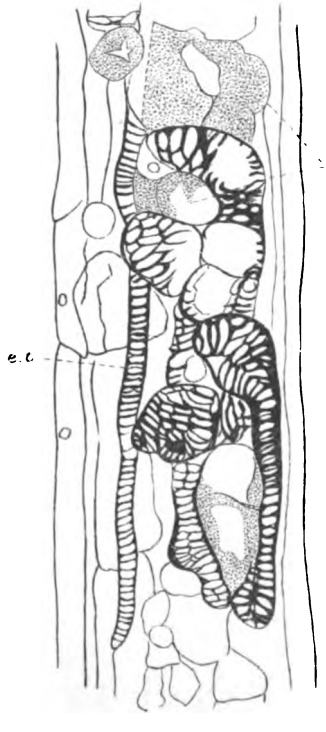
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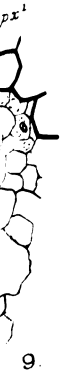

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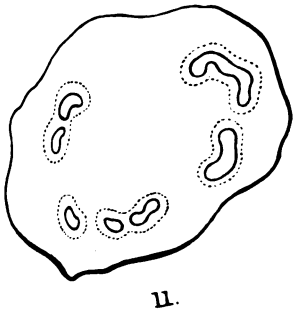

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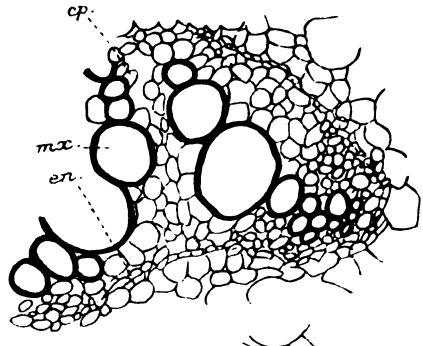

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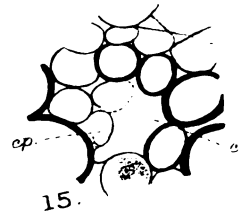

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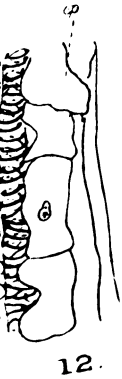

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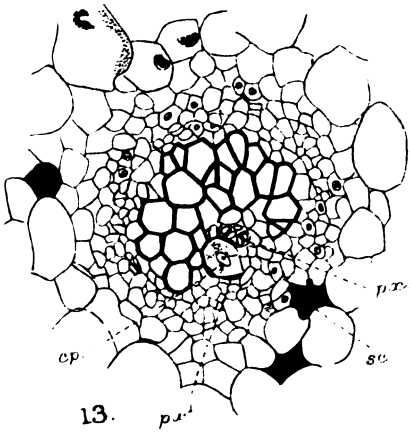

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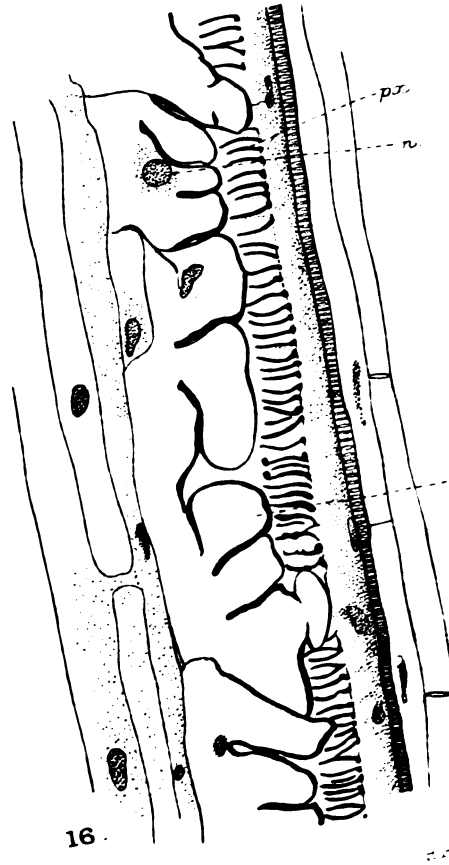

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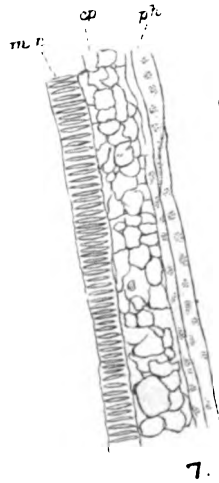

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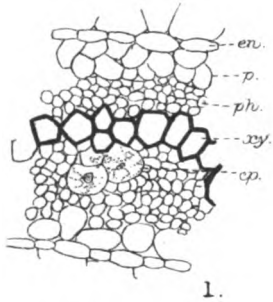
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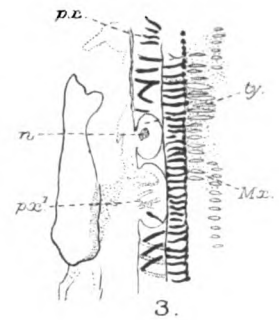
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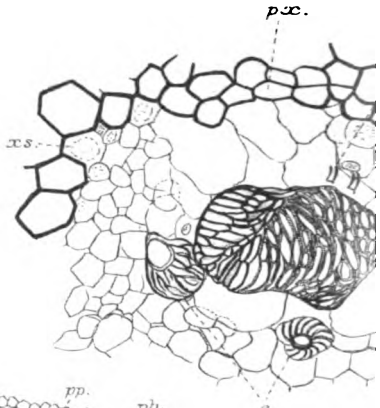
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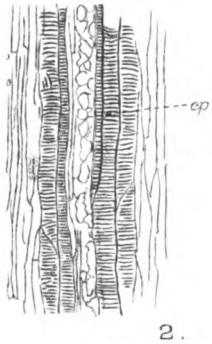
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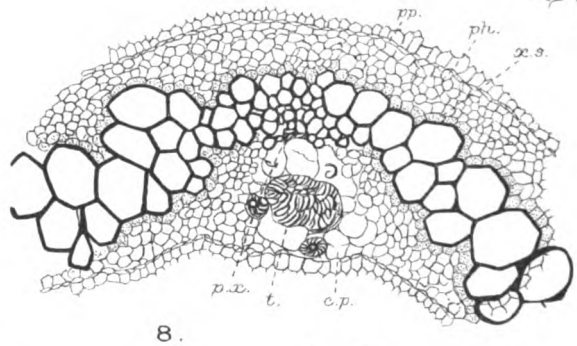
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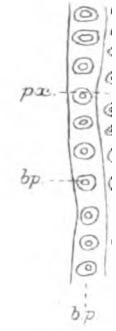
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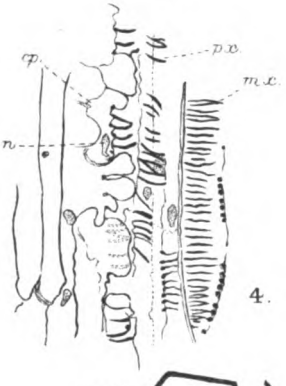
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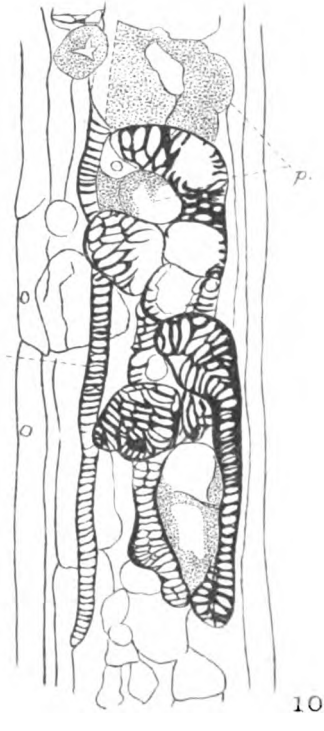
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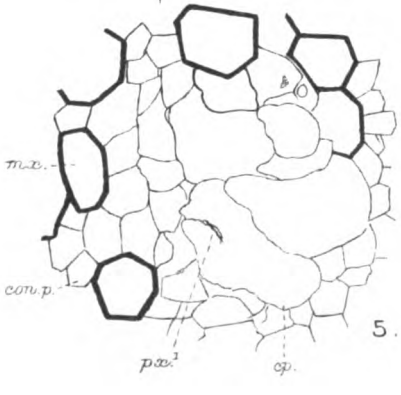
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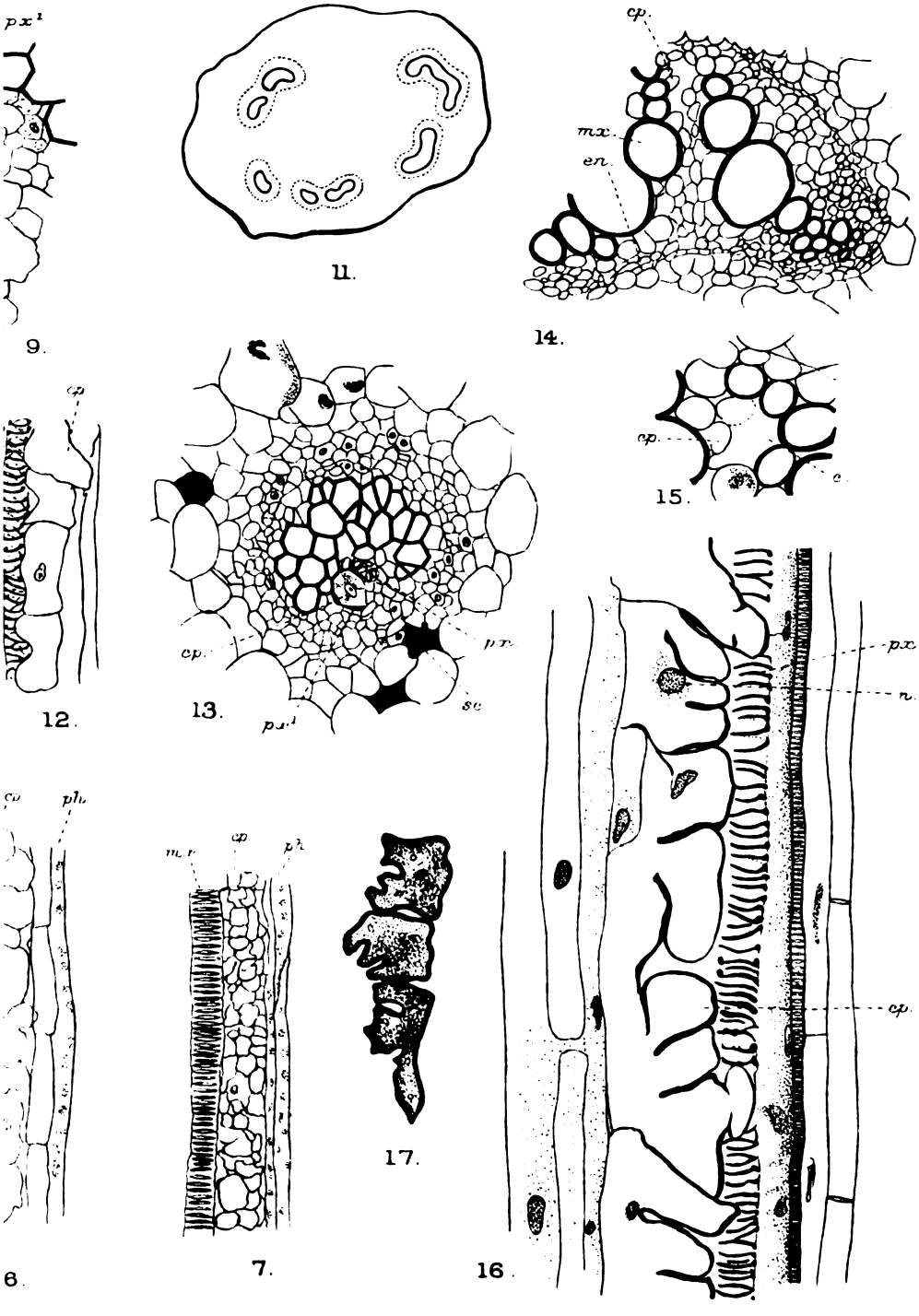

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PARENCHYMA.

On Endospermic Respiration in Certain Seeds.

BY

FREDERICK STOWARD, M.Sc. (Birm.).

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1. INTRODUCTION.

A NUMBER of experiments bearing on the nature of the amyliferous cells of the endosperm of the Gramineae have from time to time been undertaken indirectly with the apparent object of ascertaining whether these cells do or do not possess vitality.

The experimental determination of this point is a matter of considerable difficulty; these cells in the mature endosperm do not apparently undergo the usual process of cell-division when the seed is placed under conditions favourable to germination, and further the application of plasmolysing agents as a test for vitality is impracticable.

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

The view now generally accepted and recognized by Pfeffer (1), based apparently to a great extent on the work of Hansteen and Puriewitsch, is that the endosperm of the Gramineae is endowed with a capacity for auto-digestion; provided that ample provision is made for the removal of the resulting metabolic products, and favourable conditions for germination are maintained. This view evidently implies that the endosperms possess vitality.

Examination of the literature of the subject, however, shows that there is a lack of agreement on this question, different investigators having arrived at divergent conclusions in the interpretation of their experimental results.

Gris (2), in a paper dealing with the physiology and anatomy of germination, makes mention of some changes observed by him in the cell-contents of the endosperm of *Ricinus* under the influence of germination.

Van Tieghem (3), in a fuller inquiry into the subject, noticed that the isolated endosperms of *Ricinus* under favourable germination conditions were capable of respiration, and also of auto-depletion, aleurone and oil being consumed under these circumstances.

He observed that the endosperms of *Canna* (amylaceous), on the other hand, did not under similar conditions exhibit either of these phenomena.

Van Tieghem concluded that endosperms like that of *Ricinus*, containing copious reserves of aleurone and oil, capable of respiration and auto-depletion, were endowed with vitality, while those of the type represented by *Canna*, containing as reserve materials chiefly starch and cellulose, and neither exhibiting respiration nor auto-depletion, did not possess vitality.

The former he termed 'active' the latter 'passive' endosperms, and regarded the digestion of the reserve materials of the latter type as being due to the agency of the embryo.

Brown and Morris (4) subjected to an extended study the barley endosperm, and from the results of certain of their experiments advanced the view that the amylaceous endosperm of the Gramineae represented a 'dead storehouse of reserve material'. This conclusion does not apply to the endosperm as a whole, but only to the amyliferous cells, the possibility of the aleurone layer cells possessing vitality being left open.

There is thus a certain amount of agreement in this view with that suggested by Van Tieghem.

Grüss (5) germinated isolated maize endosperms under sterile conditions, and studied the distribution of diastase in them by means of the not very reliable guiac-hydrogen-peroxide test.

Hansteen's (6) conclusions are opposed to those of Van Tieghem, that self-digestion is confined to oily seeds, and that the endosperms of the grasses play an entirely passive part. One of the chief grounds on which he bases his objection is that Van Tieghem did not make sufficient

provision for the escape of the mobilized products of change derived from the supposed inactive storage-tissues of the latter.

He asserts that in the case of oily seeds, on account of the peculiar nature of the metabolism associated with them—oil first undergoing transformation into starch—the inhibitory influence of the accumulated products of self-digestion would not manifest itself so early as in the case of starchy endosperms. Hence, while auto-depletion might be observed in the case of the endosperm of *Ricinus*, it might not be in the Gramineae because of its early inhibition in their case.

He further claims to have himself demonstrated the self-depletion of the non-growing and apparently inactive endosperms of *Hordeum* and *Zea*, when placed under sterile conditions of germination, and when, also, ample opportunity was given for the removal of the products of mobilization and depletion.

Linz (7), in a study of the physiology of germination of maize, claims from the results obtained with the isolated endosperms of this seed, which were placed under germination conditions for twelve days and showed a marked increase in sugar content, that they possess vitality.

Puriewitsch (8), utilizing in the main the method of experiment devised by Hansteen, and conducting his experiments in such a manner that the absence of micro-organisms was assured, observed the corrosion of starch grains and the more or less advanced depletion of the endosperms of barley and maize.

He considers that the endosperms of the Gramineae should be regarded as living organs, and that each individual cell of the endosperm is capable of functioning autonomously as a living unit.

Brown and Escombe (9) subjected the work of Brown and Morris to a re-investigation which resulted in a demonstration of both a cytohydrolytic and an amylohydrolytic capacity in the aleurone cells of barley.

They were, however, unable to demonstrate an amylohydrolytic capacity for the amyliferous cells. Fragments of endosperm deprived of their aleurone layer, which were placed under conditions favourable for germination, and the ready outward diffusion of any soluble metabolic products, showed no difference as compared with similarly prepared ones, previously treated with saturated aqueous chloroform for a sufficient length of time to extinguish any residual vitality they may have possessed.

The general conclusion to be derived from these later experiments of Brown and Escombe on the barley endosperms appears to be that the aleurone cells are endowed with a depletive capacity, and are probably living, while the amyliferous cells possess no such capacity and probably do not possess vitality.

Quite apart from any possibility of determining whether the amyliferous cells of the endosperm-tissue of barley and maize do or do not

possess any residual vitality, it nevertheless appeared to be a matter of interest to ascertain whether this amyliferous tissue, when placed under favourable conditions, was capable of manifesting any activity which, outwardly at any rate, could be regarded as of a respiratory nature or allied to it.

The major part of the results here recorded have been obtained during June, July, August, and December, 1905, in the University Experiment House of the Edgbaston Botanical Gardens. The author is especially indebted to Dr. (now Professor) A. J. Ewart, to whom the initiation of the investigation is due, for much valuable advice and preliminary guidance up to his leaving England in January, 1906. He also desires to thank Professor Hillhouse for affording him facilities in carrying out the work, and for many suggestions and criticisms during its progress.

2. Material and Methods of Experiment.

As material for investigation the seeds of *Hordeum*, *Zea*, and *Ricinus* were employed, the latter more especially, on account of the fact that the endosperm of this seed is regarded on every hand as possessing vitality.

As the seeds of the Gramineae are almost invariably infested with micro-organisms it was deemed advisable at the outset to adopt the most efficient precautionary means of rendering the material for experiment sterile. To meet this difficulty the duration of some of the experiments was somewhat curtailed, and, in certain instances, as later experience showed, unnecessarily so. Further, recourse was had to the use of solutions of various antiseptic reagents, these being used either as preliminary steeping solutions or as rinsing reagents. In certain cases they also served as the steeping medium throughout the whole period covered by that operation. They are comprised in the following list: 3 % and 6 % copper sulphate; 0·1 % and 0·5 % mercuric chloride; 4 %, 0·4 %, 0·2 %, and 0·1 % aqueous formaldehyde; saturated aqueous solutions of chloroform and toluene.

Wherever the different reagents in aqueous solution were employed, either for steeping or rinsing purposes, the material, prior to steeping in water or the establishment of the respiration experiment, was thoroughly washed with sterilized water to wash out as far as possible all removable traces of them.

The operation of steeping in reagent solutions and in sterilized water was undertaken either in small cylindrical glass boxes with loosely fitting covers, or in ordinary securely stoppered weighing-bottles. In those experiments where a saturated aqueous solution of chloroform or toluene, or a 4 % formaldehyde solution, constituted the steeping medium, stoppered weighing-bottles were used, a fairly large bulk of liquid in relation to the bulk of material under experiment being employed. In the steepings in water, however, the volume of liquid employed was as small as possible.

The duration of the steeping operation was varied considerably. At its termination the experimental material was superficially dried, by placing it on or between folds of sterilized blotting-paper, and then quickly transferred to small tarred cylindrical respiration-tubes, having a total capacity of approximately 10 c.c., and graduated in divisions of 0·5 of a c.c. Each tube was plugged with a sterilized cotton-wool plug. The weight of the material was then ascertained, the plug at once removed, and the respiration experiment commenced by inverting the tube over mercury contained in a small dish.

Where necessary, a small quantity of water—one or two drops—was introduced by means of a small curved capillary pipette to ensure the maintenance of the material in a moist condition. At given intervals specimens of the gaseous respiration products were removed, by placing the respiration-tube in direct communication with the graduated tube of the apparatus employed for carrying out the analysis.

The gas analyses were made with the improved Bonnier-Mangin apparatus, by means of which very small volumes of gas can be subjected to analysis. This apparatus is particularly serviceable in cases where only small quantities of gas are available. For the absorption of CO_2 and O_2 a 40 % solution of KOH, and a cold saturated aqueous solution of pyrogallol diluted to one-tenth its original strength, were respectively employed. This latter solution was either freshly prepared as required, or on every second day. To avoid the decomposition of the pyrogallol with evolution of carbon monoxide (which occurs when the caustic potash solution is not used in sufficient excess), the analyses have been so performed that the ratio of the volumes of the alkali and pyrogallol solutions was 2 : 1. There is also a distinct advantage in employing these reagents in such total quantity (which can be experimentally determined) that neither is in large excess relatively to the quantities of the gases to be absorbed : the subsequent washing out of the apparatus on the completion of the analysis being thus rendered more expeditious.

3. Experiments with Seeds of *Hordeum*.

These were undertaken with a well-matured Chilian barley which consisted of a mixture of *H. vulgare* and *H. hexastichum* of the year 1904, and possessed a satisfactory germinative capacity.

The general method of preparation for intact seeds,[1] embryos, and endosperms was the following:—

Intact seeds were washed in running tap-water for a quarter to half an hour, then steeped in 3 % or 6 % copper sulphate for periods of one to three hours, and finally were thoroughly washed with, and steeped in, sterilized

[1] For convenience the term 'seed' is used throughout to imply the fruit or caryopsis of the Gramineae.

water. Respiration experiments were then carried out with these intact seeds, or with the embryos and endosperms derived from them. In certain experiments with endosperms, the embryo was removed from the seed either during the course or at the termination of the steeping operation. Endosperms and pure endosperms (a term used to denote the endosperm deprived of its spermoderm and aleurone layer), derived from the seed in the dry resting condition, were respectively prepared by degerming the dry seed, and by degerming and also filing off the spermoderm and aleurone layer. Pure endosperms from germinated seeds were prepared by removing the developed embryo (seedling) and stripping off with forceps the integuments and the aleurone layer which so closely adheres to the endosperm.

These different objects, with the exception of the pure endosperms from germinated seeds, after the termination of the steeping operation were rinsed in 0·1 % aqueous formaldehyde, thoroughly washed with sterilized water, superficially dried, and at once transferred to the respiration-tubes.

In other experiments endosperms and pure endosperms derived from dry resting seeds were steeped in saturated aqueous chloroform, and experiments were also performed with similarly prepared endosperms steeped in 4 % aqueous formaldehyde.

A number of experiments were undertaken with endosperms and pure endosperms which were subjected to diminished pressure for a short time just prior to the establishment of the respiration experiment.

The majority of the experiments were carried out in such a manner that at no time during their progress were they interrupted by the displacement of the respiration-gases in the tube by a fresh supply of normal atmospheric air, and, unless otherwise stated, this is to be inferred; in other experiments, however, this latter procedure was followed after the expiration of definite intervals of time, the experiment thus undergoing a complete re-establishment. Sections 12, 13 and 14 will show a comparison of the results under these two experimental methods.

The experimental data, for convenience of survey, are grouped under the following headings:—

1. Intact seeds.
2. Embryos.
3. Endosperms.
4. Pure endosperms.

The experiments, with these different objects, having been undertaken during two different periods of the year, viz. summer and winter, are further subdivided into 'summer' and 'winter' experiments.

In the tables which follow, the temperatures and volumes in c.c. represent those observed at the time of the removal of the sample of gas for analysis.

The numbers given under 'Vol. c.c.' are the actual volumes read off in the respiration-tube, less the volume of the experimental material as determined by its water-displacement value.

The values given for the output of CO_2 have been calculated from the percentage values found, and are stated as milligrams of CO_2 per gram of moist material per hour, and the calculation is further based on the assumption that one gram-molecule (44 grams) of CO_2 occupies 22,400 c.c. at 0° C. and 760 m.m. In these calculations the temperatures have all been reduced to 0° C., but no correction has been applied for change of pressure.

Under 'time' the number of hours are given which have elapsed from the commencement of the experiment.

The final column gives the ratio $\frac{CO_2}{O_2}$ (respiration quotient), which has been calculated from the CO_2 and O_2 percentages; it having been assumed that at the commencement of each experiment 20 % of O_2 was present.

4. Table. Experimental Data. *Hordeum.*

Summer Experiments.

Intact Seeds.

Exp. 1. Seeds steeped in water, 48 hours: paleae removed.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
20	1·57	5·5	29°	4	10·3	9·83	0·1601	1·01

Exp. 2. Seeds steeped in (1) 3 % $CuSO_4$, 1½ hours; (2) water, 23½ hours.

25	1·419	5·0	32°	4	14·23	4·92	0·2193	0·94

Exp. 3. Seeds steeped in (1) 3 % $CuSO_4$, 3 hours; (2) water, 21 hours.

25	1·479	6·75	26°	4	9·91	8·18	0·2028	0·83

Exp. 4. Seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 22 hours.

25	1·473	5·25	25°	1	2·104	17·38	0·1350	0·80
		5·10	26°	2	4·097	14·34	0·1273	0·72

Embryos.

Exp. 5. Intact seeds steeped in (1) 3 % $CuSO_4$, 1 hour; (2) water, 23 hours; embryos then excised.

25	0.117	3·5	19°	2	4·975	14·95	1·368	0·98

Exp. 6. Intact seeds steeped in (1) 3 % $CuSO_4$, 1 hour; (2) water, 22 hours; embryos then excised.

23	0·112	7·00	22·5°	1	1·237	17·67	1·403	0·53
		6·75	22·5°	2	1·521	17·30	0·8317	0·56
		6·60	21·5°	19½	17·12	2·93	0·9420	1·00

Exp. 7. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 24 hours; embryos then excised.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
30	0·118	4·5	21·5°	2	2·9	17·05	1.007	0·98
		4·25	24·0°	4½	8·56	12·16	1·237	1·09

Exp. 8. Intact seeds steeped in (1) 3 % $CuSO_4$, 1½ hours; (2) water, 18 hours; embryos then excised.

26	0·117	7·25	23°	2	2·337	17·32	1·311	0·87
		7·00	23°	3	3·457	16·25	1·249	0·92
		6·75	23°	4	5·537	14·537	1·446	1·01

ENDOSPERMS (SUMMER EXPERIMENTS).

Exp. 9. Seeds degermed in dry resting condition; endosperms steeped in water 48 hours.

20	1·27	4·75	28·5°	4	4·11	14·19	0·06835	0·70

Exp. 10. Intact seeds steeped in (1) 3 % $CuSO_4$, 1½ hours; (2) water, 5 hours; embryos then excised, and endosperms steeped in water 18½ hours.

25	1·376	5·75	27°	4	4·90	13·75	0·09150	0·78

Exp. 11. Intact seeds steeped in (1) 3 % $CuSO_4$, 3 hours; embryos then excised, and endosperms steeped in water 21 hours.

25	1·499	5·75	23°	4	3·25	14·35	0·05646	0·57
		5·5	23°	6	7·16	11·19	0·09986	0·80

ENDOSPERMS (WINTER EXPERIMENTS).

Exp. 12. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 46 hours; embryos then excised.

45	2·470	3·00	16°	4	3·601	13·54	0·02019	0·55
		2·80	15°	5⅛	5·066	11·38	0·01889	0·58
		2·50	15°	23	15·26	0·00	0·01250	

Exp. 13. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 22 hours; embryos then excised, and endosperms halved longitudinally.

42	1·781	2·1	17°	4	2·708	10·47.	0·01476	0·28
		2·1	15°	24	15·15	0·00	0·01386	—

Exp. 14. Intact seeds steeped in (1) 3 % $CuSO_4$, 1 hour; (2) water, 48 hours; then removed from steep, and laid on moist blotting-paper under bell-jar for 24 hours; embryos then excised.

39	2·438	2·8	20°	2	2·293	16·23	0·02409	0·48
		2·5	18·5°	3	4·273	13·50	0·02686	0·65
		2·2	17°	4	5·392	11·39	0·02249	0·62

Experiment re-established:

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2%.	O_2%.	*Mgms.* CO_2.	CO_2/O_2.
		2·2	17°	2	4·194	14·81	0·03499	0·80
		2·0	17°	4	7·223	9·486	0·02739	0·68
		1·8	18°	5	9·55	6·25	0·02598	0·69
		Experiment re-established:						
		3·1	22°	14¾	16·57	0·00	0·02596	

Exp. 15. Intact seeds steeped in (1) 6 % $CuSO_4$, 1 hour; (2) water, 2 hours; embryos then excised, and endosperms steeped in water 23 hours.

40	2·247	2·50	16°	5	4·34	10·45	0·01792	0·45
		2·30	17°	6	5·784	8·063	0·01824	0·48
		Experiment re-established:						
		2·60	17°	18½	16·82	0·00	0·01945	—

Diminished Pressure Experiments.

In each of the following experiments the endosperms were subjected to a pressure of 10 m.m. for 15 minutes just prior to the establishment of the experiment:—

Exp. 16. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 22 hours; embryos then excised.

30	1·81	3·25	14°	3	0·465	16·84	0·005176	0·14
		2·70	23°	23	9·824	0·877	0·01154	0·50

Exp. 17. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 46 hours; embryos then excised.

35	1·935	2·2	13°	4	1·744	15·69	0·009294	0·40
		2·0	11°	24	12·61	0·00	0·01025	—

Exp. 18. Intact seeds steeped in (1) 6 % $CuSO_4$, 1 hour; (2) water, 2 hours; embryos then excised, and endosperms steeped in water 23 hours.

32	1·870	2·6	18°	3¾	2·255	15·35	0·01541	0·47

Exp. 19. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 46 hours; embryos then excised.

40	2·215	2·70	14°	4	3·004	12·87	0·01710	0·40

Endosperms (Summer Experiments).

Material derived from dry resting seeds by degerming them, and steeped either in (*a*) saturated aqueous chloroform or (*b*) 4 % formaldehyde solution.

Exp. 20. Endosperms steeped in saturated aqueous chloroform, 24 hours.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2%.	O_2%.	*Mgms.* CO_2.	CO_2/O_2.
24	1·469	5·25	24°	4	3·02	16·44	0·04871	0·84
		5·10	24°	6	4·81	15·61	0·05025	1·05
		4·95	20°	7	5·26	15·43	0·04634	1·19
		4·80	20°	8	5·80	15·13	0·04335	1·19
		4·65	20°	9	6·13	14·54	0·03946	1·12
		4·50	24°	24	10·29	9·92	0·02317	1·02

Exp. 21. Endosperms steeped in saturated aqueous chloroform, 24 hours.

25	1·532	4·00	26°	1	2·398	17·34	0·1123	0·90
		3·80	28°	2	3·698	14·97	0·0817	0·73

Exp. 22. Endosperms steeped in aqueous 4 % formaldehyde, 24 hours.

25	1·392	6·25	25°	4	0·00	19·63	0·00
			20°	6	0·00	—	0·00
			20°	24	0·00	—	0·00

Exp. 23. Endosperms steeped in aqueous 4 % formaldehyde, 24 hours.

25	1·514	5·00	23°	24	0·00	19·51	0·00

WINTER EXPERIMENTS.

Exp. 24. Endosperms steeped in saturated aqueous chloroform, 41 hours.

40	2·65	4·00	15°	2	1·291	18·76	0·01814	1·04
		3·80	16·5°	$3\frac{3}{4}$	3·138	16·54	0·02222	0·97
		3·5	17°	5	4·127	15·73	0·02015	0·96
		2·7	17·5°	6	5·714	13·93	0·01791	0·94
		2·4	18°	7	6·582	12·79	0·01569	0·91
		2·2	18°	9	8·399	9·482	0·01427	0·79
		2·0	18°	$10\frac{1}{4}$	10·99	8·060	0·01491	0·92
		1·5	18°	$11\frac{1}{4}$	12·99	6·057	0·01204	0·93
		2·0	14°	$23\frac{1}{4}$	19·01	0·8804	0·01153	0·99

Exp. 25. Endosperms steeped in saturated aqueous chloroform, 42 hours.

40	2·640	2·5	17°	2	1·711	17·45	0·01498	0·67
		Experiment re-established:						
		2·3	17°	2	2·629	16·37	0·02117	0·72
		Experiment re-established:						
		2·8	17°	2	2·086	16·69	0·02045	0·63
		Experiment re-established:						
		2·2	18°	2	2·395	16·32	0·01839	0·65
		Experiment re-established:						
		2·2	15°	17	15·57	0·8652	0·01421	0·81

Diminished Pressure Experiments.

Exp. 26. Endosperms steeped in saturated aqueous chloroform, 24 hours; then exposed to pressure of 10 m.m. for 15 minutes.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
30	1·765	3·4	15°	4	1·383	16·60	0·01240	0·40
		2·7	24°	24	8·892	5·434	0·01070	0·61

Exp. 27. Endosperms steeped in saturated aqueous chloroform, 48 hours, then exposed to pressure of 10 m.m. for 15 minutes.

30	1·856	2·3	13°	4½	1·477	17·63	0·007626	0·62
		2·05	12°	24	7·65	6·73	0·006624	0·57

Pure Endosperms (Series 'A').

Prepared from dry resting seeds; embryos excised, spermoderm and aleurone layer filed off, and endosperm surface scraped with sharp scalpel.

Summer Experiments.

Exp. 28. Pure endosperms steeped in water, 14 hours.

50	3·00	4·25	23°	2	1·51	15·81	0·01937	0·36
		4·00	22°	4½	2·27	12·33	0·01224	0·29

Exp. 29. Pure endosperms steeped in water, 24 hours.

25	1·500	5·5	19°	0	0·00	19·37	0·00	
		5·0	20°	3	1·733	18·34	0·03524	1·04
		4·5	21°	19½	6·025	8·688	0·01741	0·54

Exp. 30. Pure endosperms steeped in water, 42 hours.

27	1·537	3·10	27°	3	1·581	17·75	0·01900	0·70
		2·90	24·5°	5½	2·389	16·04	0·01477	0·60
		2·60	28°	26	13·38	2·174	0·01550	0·75

Exp. 31. Pure endosperms steeped in water, 24 hours.

25	1·374	5·0	27°	2	1·602	18·32	0·05210	0·95
		Experiment re-established:						
		4·5	31°	2	1·95	17·91	0·05632	0·93
		Experiment re-established:						
		6·0	18°	17½	3·683	11·97	0·01693	0·45

Exp. 32. Pure endosperms steeped in saturated aqueous chloroform, 24 hours.

25	1·298	—	20°	0	0·00	19·40	0·00
		4·75	21·5°	3	0·00	19·16	0·00
		4·50	21°	5	0·00	18·39	0·00
		4·00	21°	22½	0·00	18·24	0·00

Exp. 33. Pure endosperms steeped in aqueous 4 % formaldehyde, 24 hours.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
28	1·750	4·25	26°	20½	0·00	18·91	0·00	

WINTER EXPERIMENTS.

Exp. 34. Pure endosperms steeped in water, 24 hours.

42	2·529	3·0	20°	2	0·6795	18·30	0·007376	0·39
		2·7	22°	3	1·385	17·30	0·008959	0·51
		2·4	17·5°	4	1·452	16·15	0·006359	0·37
		2·1	19°	5	1·773	15·07	0·005407	0·35
		1·9	18°	6	2·364	13·01	0·005454	0·33
		1·6	18°	7	2·683	11·45	0·004468	0·31

Exp. 35. Pure endosperms steeped in water, 23 hours.

39	2·44	3·2	14°	2	0·00	18·62	0·00	—
		Experiment re-established:						
		2·6	16°	2	0·7081	18·94	0·007611	0·68
		Experiment re-established:						
		2·9	16°	2	0·7818	18·68	0·009374	0·59
		Experiment re-established:						
		3·0	16°	2	0·3578	18·78	0·004438	0·29
		Experiment re-established:						
		1·9	18°	2	0·7018	17·54	0·005475	0·28
		Experiment re-established:						
		3·3	18°	14½	4·761	10·30	0·009054	0·49
		Experiment re-established:						
		3·0	19°	2	—	17·71	—	—
		Experiment re-established:						
		1·7	15°	2	0·965	17·28	0·006805	0·35
		Experiment re-established:						
		2·0	16°	2	0·3282	18·40	0·005677	0·35
		Experiment re-established:						
		1·9	18°	18	0·922	4·197	0·005220	0·38

Exp. 36. Pure endosperms steeped in water, 24 hours; then exposed to pressure of 10 m.m. for 13 minutes.

33	2·023	2·4	17°	4	1·344	17·47	0·005450	0·56

PURE ENDOSPERMS (SERIES 'B', WINTER EXPERIMENTS).

Material derived from germinated seeds.

Exp. 37. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 48 hours; and then germinated on moist blotting-paper under bell-jar for 3–4 days; embryo then excised, and spermoderm and aleurone layer stripped off with forceps.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
25	1·043	2·5	21°	4	1·711	16·92	0·01935	0·57
		2·25	18°	6	1·974	16·33	0·01307	0·53

Exp. 38. Intact seeds steeped in (1) 3 % $CuSO_4$, 2 hours; (2) water, 48 hours; and then germinated, as described in the preceding experiment, for 6 days; embryos then excised, and spermoderm and aleurone layer stripped off with forceps.

35	1·413	3·1	18°	3	1·901	17·47	0·02562	0·75

5. EXPERIMENTS WITH SEEDS OF *ZEA*.

The earlier series of respiration experiments with *Zea* having been undertaken with material of unsatisfactory germinative powers, it was considered desirable to practically subject the matter to a re-investigation. Hence, the work here recorded represents the results obtained with a reliable sample of the ordinary maize of commerce possessing a satisfactory germinative capacity.

While adhering to the methods of experiment already indicated under *Hordeum* for the preparation of sterile material, only the stronger (6 %) solution of copper sulphate was employed in this series of experiments, and the duration of the steeping period was considerably extended. Aqueous solutions of 0·1 % and 0·5 % mercuric chloride were also used in certain experiments in dealing with pure endosperms either for short steep purposes, or for rinsing the experimental material. Similarly, aqueous 0·1–0·4 % solutions of formaldehyde were freely used for washing purposes where their employment appeared to offer advantages. Experiments were also instituted in which saturated aqueous toluene served as the steeping medium.

Most of the experiments were performed within a comparatively small range of temperature, 17°–21° C., a modern forcing-tray, provided with a thermo-regulator, being used for this purpose.

In every instance great care was taken during the various manipulative operations to avoid accidental contamination, all these operations being carried out in a sterilized chamber.

At the termination of each experiment the material was at once inoculated into tubes of 1 % dextrose-wort, and incubated at a temperature of 20°–23° C., in order to check the efficiency of the antiseptic reagents under the specified experimental conditions.

6. Table. Experimental Data, *Zea*.

Intact Seeds.

Exp. 1. Seeds steeped in (1) 0·1 % mercuric chloride, ½ hour; (2) water, 23½ hours.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.*	*After hours of respiration*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
15	2·8802	4·7	18°	4¾	5·717	11·28	0·03619	0·65

Exp. 2. Seeds steeped in 6 % $CuSO_4$, 24 hours.

15	3·2231	4·1	18°	4¼	5·574	13·71	0·03074	0·88

Embryos.

Exp. 3. Intact seeds steeped in (1) 0·1 % mercuric chloride, ¾ hour; (2) water, 23¼ hours; embryos then excised.

10	0·5218	4·0	20°	4	11·00	5·797	0·3858	0·77

Exp. 4. Intact seeds steeped in 6 % $CuSO_4$, 24 hours; embryos then excised.

10	0·4198	5·0	20°	4	9·218	9·85	0·5024	0·90

Endosperms.

Exp. 5. Intact seeds steeped in (1) 6 % $CuSO_4$, 6 hours; embryos then excised, and endosperms steeped in (2) water, 18 hours.

15	3·3804	4·8	16°	4	1·1154	18·64	0·007346	0·82
		4·05	13°	28½	5·016	8·961	0·003954	0·45
		3·8	16°	52½	11·04	0·7833	0·004386	0·57

Exp. 6. Intact seeds steeped in (1) 6 % $CuSO_4$, 6 hours; (2) water, 18 hours; embryos then excised.

15	3·2629	4·9	16°	4	0·86	18·92	0·005991	0·79
		4·65	14°	8	2·286	17·38	0·007509	0·87
		4·15	14°	28¼	5·275	10·64	0·004437	0·56
		3·9	16°	52	11·31	0·645	0·004708	0·58

Exp. 7. Intact seeds steeped in (1) 6 % $CuSO_4$, 8½ hours; embryos then excised, and endosperms steeped in (2) water, 15½ hours.

15	3·191	3·85	21°	4	2·648	15·96	0·01457	0·65
		3·45	20°	9	6·802	9·481	0·01495	0·64
		3·20	20°	24	14·96	0·2564	0·01140	0·75

Exp. 8. Intact seeds steeped in (1) 6 % $CuSO_4$, 8½ hours; (2) water, 15½ hours; embryos then excised.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
15	3·2188	3·6	21°	4	2·908	15·87	0·01483	0·70
		3·35	20°	9	5·86	10·12	0·01240	0·59
		3·10	21°	25	15·74	0·00	0·01106	—

Exp. 9. Intact seeds steeped in (1) 6 % $CuSO_4$, 9 hours; embryos then excised, and endosperms steeped in (2) water, 15 hours.

15	2·5014	3·55	21°	4	2·189	16·09	0·01416	0·55
		3·35	21°	8	5·219	11·36	0·01593	0·60
		2·90	21°	24	13·31	0·5188	0·01177	0·68

Exp. 10. Intact seeds steeped in (1) 6 % $CuSO_4$, 9 hours; (2) water, 15 hours; embryos then excised.

15	2·9500	4·25	21°	5	2·309	16·86	0·01213	0·73
		4·00	21°	8	4·766	13·02	0·01473	0·68
		3·55	21°	24	13·69	0·2628	0·01252	0·69

Exp. 11. Intact seeds steeped in (1) 6 % $CuSO_4$, 11¼ hours; (2) water, 12¾ hours; embryos then excised, and pericarp removed.

15	2·7644	4·4	21°	8	3·623	14·21	0·01314	0·62
		4·1	21°	24	12·47	0·7833	0·01405	0·64

Exp. 12. Intact seeds steeped in (1) 6 % $CuSO_4$, 11¼ hours; (2) water, 36¾ hours; embryos then excised, and pericarp removed.

15	2·9300	4·25	21°	4	2·019	14·43	0·01335	0·36
		3·50	21°	8	4·445	12·54	0·01210	0·59

ENDOSPERMS (DIMINISHED PRESSURE EXPERIMENTS).

Exp. 13. Intact seeds steeped in (1) 6 % $CuSO_4$, 8½ hours; embryos then excised, and endosperms steeped in (2) water, 15½ hours. Prior to establishment of respiration experiment, material was subjected to pressure of 20 m.m. for 40 minutes.

15	3·0602	4·35	21°	4	2·030	16·64	0·01315	0·60
		3·85	21°	8	3·831	12·79	0·01098	0·53
		3·60	21°	23¼	10·86	1·795	0·01002	0·59

Exp. 14. Intact seeds steeped in (1) 6 % $CuSO_4$, 8½ hours; (2) water, 15½ hours; embryos then excised, and endosperms subjected to pressure of 20 m.m. for 40 minutes.

15	2·6416	3·95	21°	4	2·428	16·86	0·01655	0·77
		3·65	21°	8	5·347	12·18	0·01684	0·67
		3·35	21°	23	13·68	0·2618	0·01407	0·69

ENDOSPERMS DERIVED FROM GERMINATED SEEDS.

Exp. 15. Intact seeds steeped in water, 25 hours; then washed with 0·4 % formaldehyde, and germinated in sterilized sand at temperature of 20°–21° C. for 68 hours. Ten seeds then selected, and after amputation of their plumules and radicles these were steeped in 0·1 % mercuric chloride for ½ hour. They were then thoroughly washed with sterilized water, and their embryos excised.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of espiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
10	1·8698	4·0	20°	8	3·211	14·66	0·01571	0·61
		3·7	19°	25	8·469	2·99	0·01231	0·49

Exp. 16. Intact seeds steeped in (1) 0·1 % mercuric chloride for ½ hour; (2) water, 32 hours, and germinated in sterilized sand at a temperature of 20°–21° C. for 64 hours. After amputation of their radicles and plumules, and steeping in 0·1 % mercuric chloride for 10 minutes, followed by thorough washing with sterilized water, the embryos were excised.

15	2·7426	2·7	21°	8	6·22	11·0	0·01396	0·69
		2·5	21°	24	14·56	1·154	0·01008	0·77

ENDOSPERMS PREPARED FROM DRY RESTING SEEDS, and steeped in either (1) saturated aqueous chloroform, or (2) toluene, or (3) 4 % formaldehyde.

Exp. 17. Endosperms steeped in saturated aqueous chloroform, 24 hours.

15	3·4375	3·4	20°	8	0·00	19·14	0·00
		3·15	19°	24	0·00	19·21	0·00

Exp. 18. Endosperms steeped in saturated aqueous chloroform, 24 hours.

15	2·867	3·7	20°	8	0·00	18·73	0·00

Exp. 19. Endosperms steeped in saturated aqueous chloroform, 48 hours.

15	3·1181	4·0	17°	8	0·00	19·36	0·00
		3·75	20°	25½	0·00	19·18	0·00

Exp. 20. Endosperms steeped in saturated aqueous toluene, 24 hours.

15	3·1917	4·25	20°	8	0·00	19·29	0·00
		4·00	19°	24	0·00	19·20	0·00

Exp. 21. Endosperms steeped in saturated aqueous toluene, 24 hours.

15	2·7958	4·15	20°	8	0·00	19·56	0·00
		3·90	20°	23	0·00	19·50	0·00

Exp. 22. Endosperms steeped in saturated aqueous toluene, 48 hours.

15	2·9681	3·6	17°	8	0·00	19·04	0·00
		3·4	20°	24	0·00	18·79	0·00

Exp. 23. Endosperms steeped in aqueous 4 % formaldehyde, 24 hours.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2%.	O_2%.	*Mgms.* CO_2.	CO/O_2.
5	1·4181	4·75	19°	4	0·00	20·04	0·00	—
		4·50	20°	72	0·00	19·98	0·00	—

PURE ENDOSPERMS DERIVED FROM STEEPED SEEDS.

Exp. 24. Intact seeds steeped in (1) 6 % $CuSO_4$, 10½ hours; (2) water, 37½ hours; embryos excised, and their spermoderm and aleurone layer filed off.

15	2·7649	3·6	21°	8	2·247	18·44	0·006670	1·44
		3·35	21°	25	4·544	14·34	0·004016	0·80

Exp. 25. Intact seeds steeped in (1) 6 % $CuSO_4$, 11 hours; embryos excised, and endosperms steeped in (2) water, 13 hours; spermoderm and aleurone layer then filed off.

15	2·7467	3·80	20°	8	1·667	18·17	0·005241	0·91
		3·55	21°	24	5·467	12·46	0·005335	0·72

Exp. 26. Intact seeds steeped in (1) 6 % $CuSO_4$, 11 hours; embryos excised, and endosperms steeped in (2) water, 37 hours. Spermoderm and aleurone layer then filed off.

15	3·8160	3·40	20°	7½	1·668	17·52	0·003626	0·67
		3·10	21°	24	6·574	9·388	0·004058	0·61

Exp. 27. Intact seeds steeped in (1) 0·1 % mercuric chloride, ½ hour; embryos then excised, and spermoderm and aleurone layer filed off. Pure endosperms then steeped in (2) water, 18 hours.

15	1·6948	3·35	20°	8	0·86	18·39	0·003889	0·53
		3·05	17°	24	1·993	16·29	0·002763	0·53
		2·8	17°	32¾	3·32	14·20	0·003097	0·57

Exp. 28. Intact seeds steeped in (1) 6 % $CuSO_4$, 6 hours; embryos then excised, and spermoderm and aleurone layer filed off. Pure endosperms then steeped in (2) water, 14 hours.

15	1·878	2·7	22°	8	1·43	18·06	0·004671	0·73
		2·5	23°	24	7·092	9·915	0·007126	0·70

Exp. 29. Intact seeds steeped in (1) 0·5 % mercuric chloride, ½ hour; embryos then excised, and spermoderm and aleurone layer filed off. Pure endosperms then steeped in (2) water, 19 hours.

15	2·026	3·2	20°	8	0·00	18·84	0·00	—
		2·9	20°	24	0·00	18·26	0·00	—

Exp. 30. Intact seeds steeped in (1) 0·5 % mercuric chloride, 1 hour; embryos then excised, and spermoderm and aleurone layer filed off. Pure endosperms then steeped in (2) water, 20 hours.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
15	2·6738	4·05	18°	8	0·00	19·56	0·00	—
		3·75	20°	24	0·00	18·24	0·00	—

PURE ENDOSPERMS DERIVED FROM GERMINATED SEEDS.

Exp. 31. Intact seeds steeped in (1) 0·1 % $HgCl_2$, ½ hour; (2) water, 33 hours; and then (3) germinated in sterilized sand, 64 hours; radicles and plumules amputated, and seeds placed in 0·1 % $HgCl_2$, 10 minutes. Then, after washing with sterilized water and drying, spermoderm and aleurone layer filed off.

15	1·9194	2·85	21°	8½	0·86	18·78	0·002661	0·70
		2·35	21°	26	2·394	15·34	0·001997	0·51

7. EXPERIMENTS WITH SEEDS OF *RICINUS*.

Only three experiments were made with the endosperms of this seed, the variety employed being *R. Zanzibarensis*. The interest associated with these experiments with this seed, lies in the fact that its endosperm is universally regarded as possessing vitality.

8. TABLE. EXPERIMENTAL DATA, *RICINUS*.

Exp. 1. Intact seeds steeped in water, 19½ hours; embryo then excised, and endosperm sliced into four pieces.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* CO_2.	CO_2/O_2.
1	1·297	4·4	25°	4	11·08	1·406	0·1690	0·59

Exp. 2. Dry unsteeped seeds degermed, and endosperm sliced into four pieces.

1	0·609	—	15°	5	0·00	20·46	0·00	—
			16°	25	0·00	18·51	0·00	—

Exp. 3. Outer integument removed; seed steeped in saturated aqueous chloroform, 24 hours; embryo then removed, and endosperm sliced into four pieces.

1	0·985	2·9	14°	5	3·619	13·15	0·03983	0·52
	Experiment re-established:							
		3·7	15°	23½	12·28	1·930	0·03656	0·67

9. Influence of Temperature on Respiratory Activity.

In comparing the respiratory activity of the seed and its separate components (embryo, endosperm, and pure endosperm), the output of CO_2 in milligrams per gram of moist material has been adopted as a working criterion.

The discussion of the influence of temperature on the respiratory activity of the endosperms and pure endosperms of *Hordeum* presents considerable difficulty, because the experiments were performed under variable temperature conditions, and during the summer and winter months.[1]

The general statement may be made that, both in the 'summer' and 'winter' experiments with endosperms and pure endosperms of *Hordeum* the higher range of temperature is responsible for an accompanying increased output of CO_2. The influence of temperature on the respiratory activity, though variable, is quite manifest. The comparatively low output of CO_2 in the winter experiments with similar objects, while attributable in part to the lower range of temperature under which these experiments were performed, is also probably associated with the ageing of the seeds during the interval of time which elapsed between the performance of the two series of experiments.

The majority of the experiments with *Zea* were executed under more constant temperature conditions, and here quite safe comparisons regarding the influence of this factor may be made. Thus, if experiments 5 and 6 (endosperms) are compared with almost any of those which follow (endosperm experiments) for a time interval extending over four to eight hours, it is seen that for a rise in temperature of approximately 5° C. the output of CO_2 undergoes an increase of from 2–2½ times as great.

Of the experiments with the endosperm of *Ricinus*, one was with water-steeped material at a comparatively high temperature, the other with chloroform-steeped material at a lower temperature. The output in the latter experiment is significantly lower than in the former; in how far the lower temperature has influenced the result it is impossible to decide from the data collected. It is noteworthy that during the first respiratory interval the relation $\frac{CO_2}{O_2}$ does not vary greatly in the two experiments.

[1] The experiments with *Hordeum* and *Ricinus* were made in the University Experiment House of the Edgbaston Botanical Gardens; those described with *Zea*, by the kind permission of Professor Adrian Brown, were undertaken in the Laboratory of the Department of Brewing of the University of Birmingham.

10. Table. Comparative Average Output of CO_2.

Material.	*Mgms. CO_2 per gram material per hr.*		
	Hordeum.	*Zea.*	*Ricinus.*
Embryos. (Summer experiments)	1·2315	0·4441	
Intact seeds. (Summer experiments)	0·1689	0·03346	
Endosperms. (Summer experiments)	0·07210	0·006948 (*a*)[1]	0·0818
Endosperms. (Winter experiments)	0·01762	0·01421 (*b*)	
Endosperms. (Winter experiments: material initially subjected to diminished pressure)	0·01174	0·01438 (*c*)	
Endosperms. (Winter experiments: re-established experiment)	0·02494	0·01286 (*d*) 0·01483 (*e*)	
Material steeped in saturated aqueous chloroform :—			
Endosperms. (Summer experiments)	0·06521		
Endosperms. (Winter experiments)	0·02222		
Endosperms. (Winter experiments: material initially subjected to diminished pressure)	0·01001		
Endosperms. (Winter experiments: re-established experiment)	0·01874		
Pure endosperms (A). (Summer experiments)	0·02012	0·004819 (*f*)	
Pure endosperms (A). (Winter experiments)	0·007564	0·002661 (*g*)	
Pure endosperms (A). (Winter experiment: material subjected to diminished pressure)	0·008459		
Pure Endosperms (A). (Summer experiment: re-established experiment)	0·05421		
Pure Endosperms (A). (Winter experiment: re-established experiment)	0·006919		
Pure Endosperms (B). (Winter experiment: material derived from germinated seeds)	0·01934		

11. The Relative Magnitude of the CO_2 Output by the Seed and its Component Parts.

Separate experiments having been carried out with the intact seeds, embryos, endosperms, and pure endosperms of *Hordeum* and *Zea*, it is possible to make an approximate comparison of the relative respiratory activities of the intact seed and its component parts.

Before entering upon this subject it is desirable to point out the difference in constitution which exists between the tissues of the embryo and endosperm. The tissues of the former consist of cells with actively functioning nuclei and abundant protoplasmic contents; the great bulk of these cells are in a state of vigorous and active growth. The tissues of the latter, on the other hand, are composed chiefly of the amyliferous cells with senescent nuclei, and a greatly diminished protoplasmic network. It is the vitality of these amyliferous cells which has been challenged. Forming

[1] The terms 'Summer' and 'Winter' experiments apply only to *Hordeum*.

(*a*) Low temperature experiments.
(*b*) Higher temperature experiments.
(*c*) Material initially subjected to diminished pressure.
(*d*) Material subjected to prolonged steep.
(*e*) Material derived from germinated seeds.
(*f*) Material derived from resting seeds.
(*g*) Material derived from germinated seeds.

an almost complete envelope to the starch-bearing cells is the aleurone layer, consisting of a three-celled layer in *Hordeum*, and a one-celled layer in *Zea*. The living nature of the cells of this layer, with their rich protein and protoplasmic contents and apparently active nuclei, has been based hitherto mainly on cytological evidence.

If, as already indicated in the preceding section, our comparisons be made on the output of CO_2 per gram of moist material per hour, then the relative magnitude of CO_2 production yielded by the seed and its parts assumes the following order :—

1, isolated embryos; 2, intact seeds; 3, endosperms; 4, pure endosperms.

If average values are taken for the purpose of instituting these comparisons (*vide* Table, 'Comparative Average Output of CO_2'), then, confining ourselves to the summer experiments with *Hordeum*, the following statement may be advanced :—

1 gram of embryonic tissue respires

$\frac{1{\cdot}2315}{0{\cdot}1689}$ = 7·3 times more actively than 1 gram of intact seed.

$\frac{1{\cdot}2315}{0{\cdot}07210}$ = 17·0 times more actively than 1 gram of endosperm.

$\frac{1{\cdot}2315}{0{\cdot}02012}$ = 61·2 times more actively than 1 gram of pure endosperm.

$\frac{1{\cdot}2315}{0{\cdot}05421}$ = 22·71 times more actively than 1 gram of pure endosperm (re-established experiments).

Similarly, from the average values given for *Zea*, the following data are derived :—

1 gram of embryonic tissue respires

$\frac{0{\cdot}4441}{0{\cdot}03346}$ = 13·27 times more actively than 1 gram of intact seed.

$\frac{0{\cdot}4441}{0{\cdot}006948}$ = 63·9 times more actively than 1 gram of endosperm (low temperature experiments).

$\frac{0{\cdot}4441}{0{\cdot}01421}$ = 31·3 times more actively than 1 gram of endosperm (high temperature experiments).

$\frac{0{\cdot}4441}{0{\cdot}004819}$ = 92·2 times more actively than 1 gram of pure endosperm (derived from resting seeds).

$\frac{0{\cdot}4441}{0{\cdot}002661}$ = 166·8 times more actively than 1 gram of pure endosperm (derived from germinated seeds).

From the average values given in the table referred to, still other comparisons involving the relative outputs of CO_2 by endosperms, pure endosperms, and intact seeds may be made. In all such comparative

statements, however, it is obvious that when the endosperm and pure endosperm are considered in conjunction with the embryo, the comparison is one which is open to the objection that the attempt is being made to compare two things of very different constitution, in that the bulk of the weight of the endosperm consists of starch enmeshed in a very small quantity of protoplasm, which latter, weight for weight, may be as active as that of the embryo.

Another method of representation may be advanced to express this same feature. We may compare the relative CO_2 production furnished by an equal number of intact seeds and their component parts, selecting for this purpose experimental data which are approximately comparable in regard to duration of respiration and range of temperature, and thus consider the question from this new standpoint. The selected data are arranged in the following table:—

TABLE. RELATIVE OUTPUT OF CO_2.

Hordeum.			
Expt.	*No. of objects.*	*After hours of respiration.*	*Mgms. CO_2 per hour per 25 objects.*
(7)	25 embryos	4½	0·1216 [1]
(10)	25 endosperms	4	0·1259
(29)	25 pure endosperms	3	0·05286
(3)	25 intact seeds	4	0·2999
	Zea.		
(3)	15 embryos	4	0·3019 [1]
(8)	15 endosperms	4	0·04773
(25)	15 pure endosperms	8	0·01844
(2)	15 intact seeds	4¼	0·09907

This mode of representation leads to a change in the order of magnitude of the relative output of CO_2 for *Hordeum*; this now becomes (1) intact seeds, (2) endosperms, (3) embryos, (4) pure endosperms, while the order for *Zea* undergoes no alteration. Reference to the table readily shows what a comparatively large share of the respiratory exchanges, during the earlier stages of respiration at least, is due to the endosperm and pure endosperm in the case of *Hordeum* when regarded on this new basis—a point which can hardly be looked upon as unimportant when economically considered.

Much the same may be said with regard to *Zea*, where the output is, with the exception of the embryo, of a lower order. The large output of CO_2 by the isolated embryo of *Zea*—fully three times greater than that of

[1] Calculated values for 25 objects (*Hordeum*) and 15 objects (*Zea*).

the same number of seeds—appears to imply that the isolated embryo of this seed respires more actively than does the embryo when forming part of the intact seed. That such a result may actually occur finds some support from the following considerations:—

1. When plant-tissues are subjected to injury they manifest (10) a 'wound reaction' the outward expression of which is a marked increase in their respiratory activity.

2. The increased respiratory activity (greater CO_2 production) of the isolated embryo may, in part, depend upon the greater amount of surface directly exposed to atmospheric air, thus tending to facilitate the process of gaseous diffusion both inwards and outwards, within certain limits.

Slight wounding of the embryo of *Zea* during its removal from the seed is almost unavoidable, but to what extent both this and the second factor influence its respiratory activity must be left for more direct experimental investigation.

The difference in the output of CO_2 by the endosperms and pure endosperms of both these seeds may fairly be attributed to the respiration of the aleurone layer.

Thus, removal of the aleurone layer from the endosperm in both the 'Summer' and 'Winter' experiments with *Hordeum*, and in the experiments with *Zea*, leads to marked fall in the output of CO_2, and indicates approximately the comparatively large share of the total respiratory output that is due to aleurone layer respiration.

This difference is still more clearly placed in evidence in the following table:—

TABLE. ALEURONE LAYER RESPIRATION.

	Average output Mgms. CO_2.	*Difference = Aleurone layer respiration. Mgms. CO_2.*
Hordeum.		
Endosperms (Summer experiments) . .	0·07210	
Pure endosperms (Summer experiments)	0·02012	0·05198
Endosperms (Winter experiments) . .	0·01762	
Pure endosperms (Winter experiments).	0·007564	0·010056
Zea.		
Endosperms	0·01421	
Pure endosperms	0·004819	0·009391

12. NON RE-ESTABLISHED EXPERIMENTS.

Most of the experiments with endosperms and pure endosperms of *Hordeum*, and all of those with *Zea*, were performed in such a manner that the experiment once started was continued during its entire course without

displacement of the gaseous respiration products by atmospheric air at any subsequent stage. These have been termed 'non re-established' experiments.

They present a common feature as regards the output of CO_2, which is more or less independent of the temperature changes experienced during each experiment. In general they are characterized by an apparently high initial output of CO_2, which is succeeded by a further slight rise; and this is subsequently followed by a progressive diminution of the output as the experiment progresses. This apparently high initial output, followed by a slight rise, may perhaps be explained on the ground that, during the initial stage of the experiment in certain cases, the CO_2 had not acquired its maximum tension within the tissues, thus leading to a delay in the outward diffusion of this gas. When this tension is reached, diffusion proceeds more regularly, and then the CO_2 attains, in the gaseous space without, what may be termed its normal concentration.

The progressive diminution in the output apparently turns on the changing concentrations of O_2 and CO_2 in the gaseous medium surrounding the material; the former slowly diminishing, the latter slowly increasing; the combined effect of which is probably to produce the results observed, i. e. an increasing diminution in the output of CO_2 as the experiment progresses. Further, the CO_2, when its concentration is sufficiently great, probably exercises a retarding or poisonous action on the respiratory activity of the tissues of the endosperm, or of the respiratory enzymes which they may contain.

13. Re-established Experiments.

Certain experiments with the endosperms and pure endosperms of *Hordeum* were interrupted during their course; the gaseous respiration products, after withdrawal of the sample for analysis, being displaced by atmospheric air which was made to pass through a sterilized cotton wool filter; the experiment thus undergoing re-establishment at certain definite intervals.

The results of these re-established experiments show that, in contrast with those furnished by the non re-established experiments, the output of CO_2 does not exhibit that progressive diminution which was a common feature of the latter experiments; instead, the output under these circumstances is maintained slightly above or below a certain mean level. This fact tends to show that in these instances of endospermic respiration the process cannot be regarded as a mere physical process of diffusion of CO_2 from a storage source of this gas (the endosperm), accompanied by a simultaneous absorption of O_2; but that there is a more or less regular production of CO_2, accompanied by O_2 absorption, which is well maintained over intervals of time covering several hours.

The maintenance of this more or less constant output further tends to strengthen the view that the respiratory exchanges are due to the respiratory activity of the material under investigation, and not in part to micro-organisms which may have fortuitously gained admission either before or during the course of the experiments.

An objection which may be urged against both types of experiment (re- and non re-established) is that no provision was made to ensure uniform mixing of the gaseous respiration products before removing the specimen for analysis. When, however, it is borne in mind that the total volume of the gaseous mixture in these experiments only amounted to a few c.cs. it will be evident that, even without the aid of any mechanical device to ensure uniform mixing, lack of uniform diffusion could only occur in a very restricted sense, if at all.

14. Effect of Diminished Pressure on Initial Output of CO_2.

In a number of experiments, the material, just prior to the establishment of each experiment, was subjected to diminished pressure for a short interval of time.

As the results with endosperms of *Hordeum* in general show, the effect of subjecting material to diminished pressure tends to reduce the initial output of CO_2. The output of CO_2 observed in these experiments as they progress, instead of progressively diminishing as in the non re-established ones, tends to increase; so that at the termination of the experiment the final is rather larger than the initial output, i. e. the result is a reversal of that observed in the non re-established experiments.

The results also demonstrate that, on the one hand, when the tissue is initially depleted of part of its CO_2, an appreciable time must necessarily elapse before this gas again acquires its maximum tension in the tissues; and following on this its normal concentration in the gaseous space outside; while, on the other, they strengthen the evidence already put forward in favour of the view that an actual *production* of CO_2 takes place.

Not only is the tissue under these circumstances depleted in part of its CO_2, but also of its O_2, for in all these experiments the increased absorption of O_2 is a noticeable feature.

Similar observations apply in the main to those experiments with *Hordeum* in which chloroform-steeped endosperms were initially exposed to diminished pressure, although here a small difference is noticeable in the initial and final outputs of CO_2, the final being slightly lower than the initial. Yet, if these results are compared with those of chloroform-steeped endosperms, not subjected to diminished pressure, it will be seen that the final output, relatively to the initial, is lower than in the experiments just considered.

The influence of diminished pressure on the initial output of CO_2 in the case of *Zea* is quite undemonstrable.

15. Experiments with Chloroform-steeped Material (Endosperms and Pure Endosperms).

Dry degermed endosperms of *Hordeum*, after steeping in saturated aqueous chloroform for twenty-four to forty-two hours, are still capable of manifesting respiratory exchanges; and the relative output of CO_2 does not greatly differ from that observed in the case of water-steeped material.

Similarly the endosperm of *Ricinus* also retains its respiratory capacity, although in a diminished degree, while the endosperm of *Zea* is no longer able to function.

Pure endosperms of *Hordeum*, after twenty-four hours immersion in this reagent, were found to have completely lost this capacity.

The apparently toxic effect on the pure endosperm of *Hordeum*, and its comparatively feeble effect on the endosperm of this seed, suggested the possibility of its entry into the endosperm being retarded in some way.

To gain information on this point a series of imbibition experiments were performed with the endosperms of *Hordeum*.

The results are given in the following table:—

Imbibition Experiments.

Endosperms of *Hordeum*.

Exp. 1. Seeds degermed in dry resting condition; paleae removed as far as possible, and parallel series of endosperms steeped in (*a*) distilled water, (*b*) saturated aqueous chloroform.

Series.	*No. of endosperms.*	*Duration of steep.*	*Medium.*	*% of medium imbibed.*	*% difference in favour of water.*
'A.'	40	24 hours	Water	52·34	
	40	24 „	Aqueous chloroform	49·32	3·02
'B.'	20	24 „	Water	46·31	
	20	24 „	Aqueous chloroform	42·96	3·35
'C.'	20	48 „	Water	55·71	
	20	48 „	Aqueous chloroform	52·26	3·45
'D.'	40	48 „	Water	64·65	
	40	48 „	Aqueous chloroform	55·82	8·83

The endosperms of 'B' (steeped in aqueous chloroform) were washed with water, drained on blotting-paper, bisected longitudinally, and placed in a securely corked tube. This tube and its contents were immersed in a water bath, and kept at a temperature of 80° C. for ten minutes; on removal from the bath, quickly cooling to laboratory temperature, and uncorking, no smell of chloroform was perceptible.

A respiration experiment with these endosperms yielded the following results :—

No. of endosperms.	*Wt. grams.*	*Vol. c.c.*	*Temp. C.*	*Time.*	CO_2 %.	O_2 %.
20	1·261	3	16°	3½ hours	0·00	19·64
		27	16°	26 ,,	0·00	19·05
			15°	50 ,,	0·00	18·54

These experiments indicate that under equal external conditions less water is absorbed by endosperms steeped in saturated aqueous chloroform than in water, the difference ranging from 3 % to 8 % in favour of water.

When the concentration of the chloroform is increased, as the following experiment shows, the amount of water absorbed diminishes.

ENDOSPERMS OF *HORDEUM* STEEPED IN AQUEOUS CHLOROFORM OF DIFFERENT CONCENTRATIONS.

No. of endosperms.	*Hours.*	*Medium.*	*% of medium imbibed.*	*% difference in favour of water.*
20	24	Water	43·03	—
20	24	½ sat. aq. chloroform	41·31	1·72
20	24	Sat. aq. chloroform	40·97	2·36

It appears probable, in view of the recent work of A. J. Brown (11), that we are concerned here with the influence exerted by the selective membrane existing in the spermoderm of the barley endosperm, on the entry of the solvent into its tissues.

That some chloroform does penetrate into the endosperm, either by way of the exposed amyliferous cells at the proximal end of the endosperm, or eventually through the spermoderm and aleurone cells, is conceivable, in spite of the fact that detection of chloroform by the sense of smell fails.

Obviously it does not enter in sufficient amount to exercise any very toxic influence on either the aleurone or amyliferous cells, or, if we regard the respiratory activity of the latter as being due to residual respiratory enzymes (produced during the anti-resting period of the seed), on these; for experiments with pure endosperms of *Hordeum* clearly show that, whether their respiratory activity be due to either living functioning protoplasm or residual respiratory enzymes, or to both, no production of CO_2 ensues with this material after twenty-four hours' immersion in this medium.

In the case of the endosperm of *Zea* there appears to be every facility for the entry of the reagent in quantities which are distinctly toxic; and here no respiratory exchanges were observed.

With *Ricinus*, steeping the endosperm in aqueous chloroform (apart from the low temperature of this experiment) apparently leads to a lowering in the output.

16. Experiments with Toluene-steeped Material.

Certain experiments with the endosperm of *Zea* prepared from the dry resting seed were performed, the steeping medium being saturated aqueous toluene.

Here, as in the case of aqueous chloroform, the results were distinctly negative; so that, in all probability, toluene exerts a toxic effect on the endospermic material of *Zea* immersed in it for twenty-fours at a temperature of 20–21° C.

17. Experiments with 4% Aqueous Formaldehyde-steeped Material.

Endosperms prepared in the usual way from air-dried seeds of *Hordeum* and *Zea* steeped in this solution for twenty-four hours invariably yielded negative results.

The effect of formaldehyde in this concentration is not surprising when we consider how toxic the action of this reagent is towards living protoplasm and enzymes alike.

Different concentrations of the reagent behave quite comparably with that found in the case of aqueous chloroform, as the following experiment demonstrates:—

Endosperms of *Hordeum* Steeped in Aqueous Formaldehyde of Different Concentrations.

No. of endosperms.	*Hours.*	*Medium % aq. formaldehyde.*	*% of medium imbibed.*
20	24	0·128	39·00
20	24	0·256	33·40
20	24	0·400	32·90

Here, again, evidence is afforded of the retarding action presented by the selective membrane to the entry of water, and presumably the solute, into the endosperm tissue.

In all these instances it seems reasonable to assume that the great bulk of these toxic agents enters by way of the exposed proximal end of the endosperm, their toxicity depending on the relative amounts which enter, the effective maximal amount differing with each reagent.

18. The Possible Diffusion of Respiratory Enzymes from the Embryo and Aleurone Layer to the Endosperm during Steeping or Germination.

If the view be accepted that the respiration of vegetable tissues is partly due to the agency of respiratory enzymes, then the possibility must be granted that these bodies may be generated either by the embryo or the aleurone layer cells, or by both, and projected into the endosperm during steeping or germination of the intact seeds.

Certain experiments were performed with *Hordeum* and *Zea* which would permit this process to freely occur.

The results of the experiments with the endosperms of *Hordeum* do not lend themselves to ready comparison in this respect, but certain experiments (*Hordeum*, Summer Experiments 9, 10, 11) may be advanced to show that removal of the embryo from the steeped seed after 0, 6½, and 3 hours respectively leads to no very marked change in the CO_2 output. Similarly in *Hordeum* (Winter Experiments 15, 12, 14, in the last of which the seeds were germinated for 24 hours, and the embryos were excised after 3, 48, and 73 hours respectively), although the output is slightly higher in the last two experiments, yet it is insufficient to strongly support the view that diffusion from the embryo had actually taken place, for the results may also be interpreted by assuming that the endosperm in Experiments 12 and 14 had emerged from its latent stage.

The results of Winter Experiments 37 and 38 with pure endosperms derived from germinated seeds, if compared with those already mentioned, or with those of Winter Experiments 34 and 35, do appear to furnish evidence of diffusion either from the embryo or aleurone layer cells, or both; for the values obtained for the output of CO_2 are greater than those for the pure endosperm in other experiments, and approach or exceed the values obtained with endosperms in Experiments 15 and 12. It is very difficult to decide, however, whether these results are due to the diffusion of respiratory enzymes, or to the emergence of the tissue from its latent period.

The experimental results with *Zea*, on the other hand, yield no evidence favouring this view of possible respiratory enzyme diffusion from the embryo or aleurone cells during steeping or germination.

19. The Respiratory Quotient $\left(\frac{CO_2}{O_2}\right)$.

The relation $\frac{CO_2}{O_2}$ given in the tables of experimental data furnishes values which throw some light on the character of the respiration of the seed and its component parts, although it must be admitted that the

following summary of the results is to be accepted in very general terms only.

If the duration of the experiment is sufficiently prolonged in the case of embryos and intact seeds of *Hordeum* and *Zea* the relation $\frac{CO_2}{O_2}$ invariably approaches unity. This is exemplified in the experiments with the embryos and intact seeds of *Hordeum* and *Zea*.

The endosperms of *Hordeum*, on the other hand (excluding certain experiments with chloroform-steeped material), even under varying temperature conditions, yield values which are invariably less than unity, and this applies also to those experiments in which re-establishment took place. The effect of diminished pressure on the respiratory quotient is very marked, the values obtained being universally low.

The values yielded by the endosperms of *Zea* are practically of the same order as those furnished by similar objects of *Hordeum*.

The results yielded by the chloroform-steeped endosperms of *Hordeum* are apparently quite distinctive, for in both the Summer experiment (20) and Winter experiment (24) the mean value for the two experiments closely approaches unity. In both experiments the number expressing the respiratory quotient is well maintained throughout. In others (21 and 25) it shows fluctuations and lies below unity.

In the experiments with pure endosperms of both seeds, just as with endosperms, there is considerable variation, but the general tendency is for the quotient to assume a value less than unity.

There thus appears to be a distinct, if variable, difference between the character of the respiration of the embryos and intact seeds on the one hand, and of the endosperms and pure endosperms of these two seeds on the other.

In making these calculations the assumption has been made that 20 % of O_2 was present at the commencement of each experiment.

20. Antiseptic Steeping Solutions: Certain Effects Observed.

Two antiseptic reagents were chiefly employed in the experiments with *Zea*, for the preparation of material (endosperms and pure endosperms), in a sterile condition for subsequent use in the respiration experiment.

Intact seeds were steeped either in a 6% $CuSO_4$ solution for six to twenty-four hours, or in a 0·1 % or 0·5 % solution of mercuric chloride for half to three-quarters of an hour at a temperature of 20°–21°C.; and, as previously described, the steeping of the seeds or the endosperms and pure endosperms derived from them was continued for a further period in sterilized water, so that the total steep in the antiseptic solution and in water covered a total period of twenty-four hours.

A number of preliminary experiments were undertaken with seeds steeped in these solutions for varying periods of time, in order to ascertain whether their germinative capacity was impaired by this treatment; the seeds after removal from the solution, thorough washing, and further steeping in sterilized water, as above stated, being divided into two series, one of which was germinated in sand and the other inoculated into tubes of 1 % dextrose wort. Control series of seeds steeped in sterilized water and subjected to similar germinative tests were also undertaken. The temperature of germination and incubation was maintained at 20–21° C.

The results yielded by the series which had been steeped in the copper sulphate solution for six to nine hours, and then placed under germination conditions, indicated that this treatment had a detrimental effect on radicle development, causing either the feeble development or almost complete suppression of this organ; whereas the growth of the plumule was as vigorous and extensive as in the control series.

Prolongation of the steeping period in the copper sulphate solution to twenty-four or forty-eight hours produced much more marked effects, fully 50 % of the seeds failing to germinate, and the remainder only feebly so. Under these conditions visible penetration of the copper sulphate, demonstrated by its blue colour, particularly at punctured or damaged parts of the integuments of the seed, and also in the neighbourhood of the radicle, was observable.

In similarly conducted experiments with 0·1 % mercuric chloride, the steeping in this solution being confined to half to three-quarters of an hour, and subsequently extended to a total period of twenty-four hours in water, the seeds germinated as readily and vigorously as in the control. When, however, 0·5 % mercuric chloride was substituted, under otherwise precisely similar conditions, only 80 % germinated normally, and of these 20 % very feebly; the balance failed to do so.

The results furnished by the inoculations showed that the series which were steeped in the copper sulphate solution for periods of only six hours remained sterile for four to five days; only scanty isolated mould-growths, attached to some parts of the surface of certain of the seeds, usually in the neighbourhood of the radicle, then made their appearance. Those steeped in mercuric chloride remained absolutely sterile until the conclusion of the experiment, i.e. after the lapse of fourteen to sixteen days.

In the series of supplementary experiments with *Zea* which were subsequently undertaken, the material at the termination of each experiment was directly placed in tubes of 1 % dextrose wort; with embryos, intact seeds, and endosperms, similar results to the foregoing were obtained. With pure endosperms, however, greater difficulty was experienced, yet, as the result of many attempts, even with these the attainment of a sterile condition during the course of the respiration experiment was amply assured.

In order to show how marked is the difference in the results obtained with sterile and non-sterile material, the following experiment with pure endosperms, which is contemporaneous with Experiment 28, and is an example of one that failed, may be adduced. The method of preparation was precisely the same as in Experiment 28.

Experiment. Material prepared precisely as in Experiment 28. Steep water at termination of steeping found to be contaminated with micro-organisms.

No. of objects.	*Wt. grms.*	*Vol. c.c.*	*Temp. C.°*	*After hours of respiration.*	CO_2 %.	O_2 %.	*Mgms.* Co_2.	CO_2/O_2.
15	1·9764	3·25 3·15	22° 23°	8½ 26¼	11·75 18·55	6·017 0·00	0·04132 0·02135	0·84

As comparison shows, during the first interval the output of CO_2 in the above experiment amounts to nearly ten times that furnished by the pure endosperms in Experiment 28 conducted under sterile conditions.

21. Experiments to check possible Errors due to Apparatus.[1]

For the purpose of checking any error due to the Bonnier-Mangin (Aubert, 12) apparatus in the analyses of the respiration gases, the gas evolved in one of the experiments with *Hordeum* was transferred to another tube, and three separate analyses of it were made.

The results are embodied in the following table:—

Table. Check Analyses of Respiration Gas.

Vol. of gas. c.c.	*Temp. C°.*	CO_2 %.	O_2 %.	CO_2 % *reduced to* 0° *C.*	O_2 % *reduced to* 0° *C.*
3·5	27°	7·791	13·41	7·090	12·20
3·25	28°	7·771	13·43	7·048	12·18
2·90	28°	7·891	13·38	7·157	12·13
			mean =	7·098	12·17

[1] Preliminary experiments with this apparatus, by means of which analyses of the air of the Experiment House were made, brought to light a source of error due to the mercury then used being contaminated with a small quantity of zinc, which readily oxidizes in atmospheric air. Thus the volume of air drawn into the calibrated tube of the apparatus underwent a diminution which could not be attributed to slight changes of pressure or temperature. When the mercury was subsequently shaken up with a solution of mercurous nitrate acidified with nitric acid and then washed with water and dried by filtering through a dry filter, this difficulty disappeared.

All the experiments with *Hordeum* and *Ricinus* were carried out with the original calibrated tube supplied by the makers of the apparatus. This, prior to the experiments with *Zea*, was damaged, and had to be replaced by a locally made tube. On examination of this tube, it was found that there was a constant minus error in the determination of the CO_2 percentage of 0·86 %, and consequently in all the CO_2 analyses in the experiments with *Zea* this amount has been added. Apparently the error was due to the bulb of the calibrated tube being irregular in shape, thus causing a small quantity of caustic potash solution to be persistently retained in it.

In each instance the deviation from the mean value is comparatively small.

The determination of the oxygen percentages has not presented any difficulty; in every instance where oxygen was the only gas remaining for analysis, as, for example, in the experiments with material steeped in aqueous chloroform, toluene, or formaldehyde, the difference in the percentage composition at the commencement and end of the experiment may be regarded as being due to the passive absorption of the gas by the material.

In the earlier series of experiments with chloroform-steeped endosperms of *Hordeum* it was recognized that the subsequent complete washing out of this reagent might be difficult to accomplish, or, on account of its decomposition under the influence of light yielding chlorine and hydrochloric acid, the analysis of the CO_2 percentage might be vitiated to some extent. In order to settle this point the following direct experiment was made. A mixture of 20 volumes of air and 7 volumes of saturated aqueous chloroform was introduced into the calibrated tube of the apparatus, thoroughly mixed, and then appropriate quantities of the absorbent solutions were added.

The result of this experiment is given in the following tabular statement, and shows that no decrease in volume occurs, which would render the percentage result for CO_2 too high; on the contrary, a slight increase ensues, which is in agreement with the fact that when chloroform is treated with a 40% solution of KOH evolution of CO_2 takes place:—

Vols. of air and saturated aqueous chloroform introduced into apparatus.	*Vols. of gaseous mixture after mixing.*	*Vol. after addition of KOH solution.*	*Vol. after addition of pyrogallol solution.*
20·4 air 7 aq. chloroform	22·0 mean =	22·45 22·50 22·47	18·30 18·30 18·30

Conclusion.

From the results which the investigation has furnished the following summary appears to be justified:—

1. When the pure endosperm tissue of both *Hordeum* and *Zea*, prepared in a manner which precludes the possible diffusion of respiratory enzymes from either the embryo or aleurone layer, is placed under appropriate conditions, it is capable of manifesting a gaseous exchange of a respiratory character. Whether this manifestation of respiratory activity is due wholly or in part to the vital activity of living protoplasm, or to the

agency of respiratory enzymes, the genesis of which occurred before the seed entered on the resting period, remains undecided.

2. The evidence of the possession of vitality by the aleurone layer, hitherto based chiefly on cytological and enzymic investigation, is reinforced by the results here recorded.

UNIVERSITY BOTANICAL LABORATORY, BIRMINGHAM.
March, 1908.

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Researches on the Vitality and Self-digestion of the Endosperm of some Graminaceae.[1]

BY

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Argument and Literature.

TWO different methods have been followed in order to solve the question whether the reserve material contained in the endosperm of amyliferous seeds is exclusively digested by enzymes secreted during germination, or whether the endosperm cells renew their vital activity and themselves dissolve their own food material. The first method consisted in seeking whether the embryo really secretes enzymes; the second in testing whether the embryo can develop and live if removed from the endosperm and provided with food artificially.

Van Tieghem ('73) and Blociszewski ('76) found that several embryos isolated from their endosperm developed when placed on starch jelly, but they took no account of the bacteria which had no doubt infected their cultures and had dissolved the starch by means of their powerful diastase. The same objection applies to the researches of Brown and Morris ('90), and Grüss ('94), according to whom the isolated embryo of Barley develops on, and liquefies, starch jelly.

Linz ('96) succeeded in making sterile embryos of Maize grown on a jelly containing soluble starch, and he found that by means of the scutellum they absorbed and secreted sugar, but did not liberate the slightest trace of diastase. Grüss confirmed this in a later paper ('97).

The epithelium of the scutellum, then, is not a diastase-secreting gland, although its cells grow enormously in length during the endosperm evacuation, and they show all the characters of vigorously secreting cells, as the cytological researches of Reed ('04), Sargant and Robertson ('05) have demonstrated. This last fact can be explained even if these cells do not liberate enzymes, for, as Linz has shown ('96), they produce most of the amylase for the nutrition of the embryo, and absorb and elaborate,

[1] From the Physiological Laboratory of the University Botanic Garden, Rome (1905–1906). (This paper is an abstract of several preceding papers, quoted at the end.)

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

as Brown and Morris have already proved, all the digestion products of the endosperm.

If the embryo does not furnish diastase to the endosperm, one must needs think that the latter dissolves its own contents. This brings us to a series of researches which I intend to complete and enlarge with my own work.

Sachs ('62) held that the endosperm of Gramineae remained quite passive during germination, and was actively exhausted by the embryo.

Gris ('65) had noted the different behaviour of amylaceous endosperms compared with those containing aleurone or oil as reserve material.

The first experiments were made by Van Tieghem ('77), who found that isolated endosperms of *Ricinus communis*, placed under conditions comparable to germination, respired and digested themselves till the aleurone and oil were consumed, while the starchy endosperm of *Canna*, and the horny endosperm of Date [1] remained passive and unchanged. This indicated that endosperms containing aleurone and oil digest themselves, and hence are living, while amylaceous endosperms and those containing hemicellulose as reserve material cannot digest themselves and so do not possess vitality.

Brown and Morris, after an extended study on the endosperm of Barley, agreed with Van Tieghem that the endosperm of Gramineae is a 'dead magazine' of reserve material. Ultimately they found that the diastatic capacity of the scutellum cells is destroyed by treatment with chloroform vapour.

They say that the endosperm cells are dead, although they contain diastase, since they later become dissolved. Certainly, Brown and Morris experimented exclusively with Barley in which, long before the starch is liquefied, the walls of the endosperm cells are totally destroyed, so that one could hardly designate such cells as living; nevertheless, in Maize grains the scutellum does not emit diastase,[2]—so, in this plant at least, the endosperm cells must produce it on their own account. Here is already a difference between Maize and Barley.

Haberlandt ('90) maintained that the aleurone layer alone secretes the whole of the diastase which dissolves the endosperm reserves, based on the fact that starch grains are attacked within twenty-four hours when placed on the isolated and washed aleurone layer. He prevented the advent of any amylase from the scutellum by interrupting the communication between this organ and the aleurone layer by means of a circular incision. But these experiments prove only that the aleurone layer also contains diastase. In fact, Linz has demonstrated that diastase occurs in the aleurone layer of Maize, which increases during germination as it is

[1] For 'digestion of Dates,' see Vinson ('07).

[2] The contrary affirmations of Laurent ('00) seem to deserve confirmation.

increasing in the endosperm ; but the quantity of ferment produced by the scutellum is always much larger.

With great skill Pfeffer ('93) and his scholar Hansteen ('94) approached the subject. They did not exactly accept Van Tieghem's statements as to autonomous evacuation occurring only in oleaceous seeds and not at all in amylaceous ones. Van Tieghem had not provided for the elimination of the products of digestion, the accumulation of which would entirely prevent further digestion, as might be easily foreseen, by the principle of mass-action which governs all reactions in chemical equilibrium.

In so far as reversible reactions are concerned, as soon as the products of decomposition have reached a certain concentration the velocity of reaction in the sense of decomposition tends to become zero, while the inverse reaction, the reconstitution of starch, becomes perceptible.

By Pfeffer's advice, Hansteen, in order to obtain the rapid removal of the starch dissolution products, fixed isolated endosperms on small plaster columns immersed almost to the top in a sufficient quantity of water. The endosperms were well aerated, and the starch dissolution products, issuing from the endosperm from the wounded side (whence the embryo had been cut off), diffused through the plaster into the surrounding liquid. In this manner a complete evacuation was secured, with removal of the reducing sugar from the starchy endosperms of Barley, Wheat, and Rye ; the process was almost complete in Maize, in the horny endosperm of Date (hemicellulose), and in other reserve tissues. If the quantity of water was limited, the evacuation was arrested almost as soon as it began.

From these results Hansteen concluded that even mealy and horny endosperms are alive, only a necessary condition for their emptying is the continuous export of the products of dissolution.

Grüss also observed ('95) that the products of the diastatic decomposition of starch paralyse the amylase action.

Linz ('96) repeated these experiments with Maize endosperms, putting them into a moist space, but so that nothing could be sent off, and he found an increase of diastase.[1] The same result was obtained by keeping endosperms for five days on damp blotting-paper.

Grüss ('96) found that sterile endosperms of Maize, kept for twelve days under conditions comparable to germination, gave a very positive reaction with hydrogen peroxide and guajacum. He obtained a similar result with Barley endosperms. If the guajacum test was available for demonstrating diastase, it could be argued that Maize and Barley endosperms form enzymes independently from the embryo. But the guajacum test is only workable for the oxidase, whose presence is known in the flour as well, that is, in evidently dead endosperm material.

By placing endosperms on little plaster columns in 80 c.c. water, Linz

[1] Brown and Morris had found that, under such conditions, diastase does not increase.

could not observe any corrosion of the starch grains at first. He also stated that diastase does not diffuse in the liquid, but that it continues to increase slowly up to the eighteenth day, so that, at the end, the starch grains are partially corroded and dissolved. The corrosion begins about the eighth day and increases till the eighteenth, as happens in normal germination. The increase of diastase in the isolated endosperm may be regarded as a proof of the vitality of this tissue stronger than that afforded by Hansteen's experiments, because, as Linz observes, even if the question were one of dead amylases, one would still have the phenomena on which Pfeffer insists. Haberlandt had previously remarked the importance of removing the products of amylosis, because he had noticed that if the embryo of Rye was cut off right to the scutellum no evacuation occurred, while it did happen if a stump of growing root was left. Under similar conditions Haberlandt only obtained an incipient dissolution in Maize, and he explained it by admitting that, in spite of the supposed secretion of diastase by the nitrogenous layer, the isolated endosperms did not empty themselves in his experiments.

Linz objects that it is not true, as Haberlandt states, that the dissolution begins from the periphery in all reserve organs, and he observes that the detached aleurone layer corrodes starch because it contains broken cells which allow diastase to diffuse out. Hansteen had, in the meantime, found that endosperms deprived of the aleurone layer and fixed on plaster, dissolve their starch. So, as Linz observes, one could believe that amylase had already migrated from the aleurone layer, had Linz himself not shown that diastase also increases in the isolated endosperm.

When Grüss, in a series of papers ('93, '94, '95, '96), attempted to support Haberlandt's views, and rather brought confusion than light into this intricate question, Puriewitsch ('98) by Pfeffer's advice, repeated Hansteen's experiments on Wheat, Rye, Barley, Rice, *Tetragonolobus purpureus*, *Phoenix dactylifera*. He put them upon little plaster columns immersed in 200 c.c. water, with the addition generally of a cubic centimetre of 5 per cent. solution of phosphoric acid, or in a solution of ·5 per cent. KH_2PO_4. Thence followed a notable corrosion of the starch grains, and the reducing substances diffused in the external liquid. The experiment was carried on for several days at 25°–27° C. The starch corrosion began near the scutellum and proceeded towards the middle of the endosperm. According to Puriewitsch the temperature hastens the evacuation because it increases respiration and accelerates the action of the diastase. Also he noticed that an accumulation of digestion products prevents the final emptying; hence he generally used a considerable quantity of water. In the outside liquid reducing substances were found, which increased when the liquid was boiled with dilute sulphuric acid. The quantity of non-reducing sugar gradually lessened as evacuation went on; hence he thinks

an easily soluble sugar, already formed, at first comes out from the endosperm, and afterwards the sugars formed by the decomposition of starch.

Sugars, and salts like KNO_3, $CaCl_2$, and KCl, at higher concentration than 5 per cent., stopped the evacuation in Puriewitsch's experiments. He explains this arrest by assuming a partial plasmolysis, which impedes the exosmosis of substances from the cell, a view which conflicts with several experiments made with yeast, in which plasmolysed cells even secrete invertase.[1]

Besides, Grüss had already made use of plasmolysis in order to cause exosmosis of diastase from germinating seeds. Also Puriewitsch has not taken into account that certain other salts, especially calcium phosphate, and even plaster itself, accelerate the activity of amylase.

The chief argument for vitality brought forward by Puriewitsch is the action of narcotics. According to our author, chloroform and ether prevent the evacuation of Maize endosperms. Of less value for the demonstration of the vitality of the endosperm cells is the observation of Puriewitsch that endosperms which are not well aerated do not empty. Indeed, if by chance we find amylase in the resting seed, chiefly in the form of proenzymes which become active by oxidation during germination, we have a similar result. On the latter point one remembers that some years ago Baranetzky ('78), Wortmann ('82), and Detmer ('83) proved that free oxygen is necessary for the formation of diastase in seeds. In short, the very experiment which ought to be the most convincing yields to Puriewitsch a result contrary to his views. Various living reserve organs after artificial evacuation fill up again if placed in concentrated nutritive solutions; but with starch and horny endosperms this did not succeed. Notwithstanding this, Puriewitsch and Pfeffer ('97) maintain that amylaceous and horny endosperms are alive, and that their evacuation is a purely vital process.

Brown and Escombe ('98, p. 14) have confirmed the fact that Barley endosperms soaked in chloroform water for twenty-four hours did not show any solution for several days, at the end of which time they were invaded by moulds and bacteria, while non-chloroformed endosperms showed a considerable evacuation. The authors observe that in this case the solution was sub-aleuronic, and they endeavour to demonstrate that only the aleurone cells can be alive.

Moreover, the cytological study of the endosperm cells indicates to these authors that, while the cells of the aleurone layer have a well-defined nucleus and all the usual marks of living cells, the starch-containing cells of the endosperm have deformed nuclei and a structure like that of aged cells. Brown and Escombe also deny the auto-digestive capacity of the amyliferous cells, because Barley endosperm, deprived of embryo and aleurone layer, and kept under conditions favourable for germination and for the removal of the

[1] Pantanelli ('06), p. 16.

products of starch dissolution, did not show any difference when compared with chloroformed endosperms. Hence they concluded that although the peripheral layer of the endosperm, the so-called aleurone layer, without doubt consists of living cells, proof could not be obtained of the existence of vitality in the starchy cells which constitute the greater part of the endosperm. However, they admit that the isolated endosperm of several Gramineae and of the Date is capable of emptying itself, provided a continuous removal of the decomposition products is assured. But this fact, in Brown and Escombe's estimation, does not suffice to show that a tissue is alive.

Recently Pound ('06, p. 181) found that the isolated endosperm does not dissolve itself, contrary to the affirmations of Hansteen and Puriewitsch.

My own researches have for a starting-point Puriewitsch's experiments, which I have carefully repeated. But I have not rested content with his methods and arguments, but have used various methods to ascertain if there is vitality, and if so how much vitality, in the endosperm of the four principal grains—Maize, Wheat, Barley, and Rye.

I began by studying the endosperm evacuation in the entire germinating seed, in order to compare it with the artificial emptying of isolated endosperms.

In the latter case the seeds, after being soaked for forty-eight hours in fresh water, were disinfected externally in 3 per cent. copper sulphate; then, after being deprived of the embryo and scutellum, they were put in Koch's boxes on little plaster columns or platforms, which were sterilized and plunged into sterile tap or distilled water or in $\frac{1}{1000}$ phosphoric acid.

The experiments were made both in free air and in chloroform atmosphere. After every experiment, portions of the material were fixed in different fixing media.

Maize.

The endosperm of Maize, in addition to the aleurone layer, shows a peripheral portion rich in protoplasm with the consistency and aspect of a horny tissue (glutinous portion) and a central part poor in protoplasm and of mealy consistency (farinaceous portion). In the isolated endosperms complete evacuation never occurs, but only a total destruction of the farinaceous portion and of a part of the glutinous mass, with very strong corrosion of the starch grains and diffusion of reducing sugar and other products of digestion (albumins) in the surrounding liquid.

The varieties of Maize used in these experiments were:—*Zea Mais*, var. *romana*, *Quarantino Mais*, *Zea Mais saccharata lilacina dulcis*, *Zea Mais saccharata rubra dulcis*.

Quarantino Mais gave the best results.

The greatest evacuation occurred about the 16th–18th day, as in the normal germination of the whole seed. Chloroform does not entirely stop, but greatly retards, the self-emptying of the endosperm.

Having obtained the latter result, one wished to see if amylase could, at the expense of a pre-existing zymogen, increase in the endosperm cells even when every trace of vitality in these was utterly destroyed, which would give rise to similar results to those spoken of above.

I give some experiments in full:—

I. 100 seeds of *Mais quarantino* (dry weight 30·5 gm.) after forty-eight hours' soaking were deprived of scutella and embryos, and were then well pounded and mixed with 100 c.c. 20 per cent. glycerine and some chloroform. Juice A.

The scutella and embryos were pounded separately with similar additions of glycerine and chloroform. Juice B.

The juices were left to digest at 18° C. in darkness. From time to time the reducing sugar and amylase were determined, the latter by treating for one hour at 56° C. 10 c.c. of juice with 10 c.c. 2 per cent. soluble starch —prepared after Lintner's method ('86)—with the addition of 1 c.c. of $\frac{1}{10}$ hydrochloric acid.

Date.	*A.*		*B.*	
	Sugar in 5 c.c.	*Amylase of 5 c.c.*	*Sugar in 5 c.c.*	*Amylase of 5 c.c.*
	CuO mg.	CuO mg.	CuO mg.	CuO mg.
April 13	17	traces	72	42·5
„ 17	67	2–3	130	51·0
„ 30	30	219	432	684·0

II. 100 seeds of *Quarantino Mais* (dry weight 29·28 gm.) were deprived of the scutella and embryos after forty-eight hours' soaking. The endosperms were well pounded with the addition of 25 c.c. glycerine, and 25 c.c. chloroform water. Juice A.

The scutella and embryos were treated separately in the same way. Juice B. Digestion at 20°–25° C.

Date.	*A.*		*B.*	
	Sugar in 5 c.c.	*Amylase of 5 c.c.*	*Sugar in 5 c.c.*	*Amylase of 5 c.c.*
	CuO mg.	CuO mg.	CuO mg.	CuO mg.
May 4	trace	20	trace	9
„ 11	184	98·8	113·52	141·9
June 5	116·96	182·8	89·44	19·68

Thus one sees there was a constant increase of sugar and amylase in all juices; only during the first day was the synthetic power of the enzyme predominant. Indeed, reducing sugars were not found in the

hydrolytic test, and preformed sugars almost disappeared—a new and important fact to which I shall return in another paper. The synthetic action of the amylase of Maize is greater for the enzyme of the endosperm than for that of the embryo, in which it may be entirely lacking (Exp. II); it exists only in the early days of evacuation, and afterwards it is overwhelmed by a powerful hydrolytic action which steadily increases. One can therefore maintain that amylase exists in the endosperm as a pro-enzyme or zymogen which becomes active during germination, possibly owing to contact with the air or through the action of cellular acids. It is to be noted that Reychler ('89), and Lintner and Eckhardt ('90) had observed after treating the gluten of Gramineae with dilute acid that it acquires a weak diastatic power; it is evident that this gluten held fast a small quantity of pro-amylase.

So we can no longer hold the view that the increase of diastase in the isolated endosperm constitutes a proof of the vitality of its cells, and one must turn to various methods of cell physiology.

At first, use was made of the plasmolys method, which gave very uncertain results, because the abundance of reserve material accumulated in the cells prevented accurate observation; but a trace of plasmolysis seemed to occur in the cells of the horny portion of the endosperm. On the contrary, by using the method of 'vital staining' (staining of living tissues) with methyl violet or aniline blue, one obtained a deep coloration of the whole farinaceous part and of some of the cells of the horny layer which lay most distant from the aleurone layer, while most of the cells of the horny portion, especially near the aleurone cells, did not take the stain; the gradual passage from the living cells occurring at the periphery of the endosperm to the dead cells in the middle was very instructive.

Nuclear staining was attempted by different methods. Those lying near the aleurone layer stain easily with iodine green, methyl green, and eosin; they are less deformed and smaller, while the deformation and resistance to the stain increased in the farinaceous portion. So we cannot say that the nuclei are totally dead, but it is proved as a matter of fact that they will absorb none of those stains which are rapidly taken up by young and living cells. The nucleus is frayed and vacuolated, and shows increasing decrepitude from the periphery to the middle of the endosperm.

Barley.

All the above experiments were carried out with Barley (*Hordeum distichum*), and show that in the Barley endosperm evacuation, if not complete, is much greater than in Maize. $\frac{1}{1000}$ phosphoric acid again facilitates the endosperm emptying, while chloroform has a much less deterrent action than upon the Maize endosperm. These facts all indicate that the vitality of the Barley endosperm must be less than that of Maize.

It is to be remarked that Puriewitsch performed his narcotic experiments only with *Cinquantino Mais.* For the rest, evacuation in Barley goes on much more rapidly than in Maize, and, as the cell-walls are attacked before the starch, it would be difficult to hold that a tissue which no longer exists as a tissue dissolves its reserves by its own vital activity—a conviction that has already taken possession of Brown and Morris.

In this case also search was made for a pro-enzyme which would become active even in aseptic autolysis at low temperatures. The conclusion was reached that a zymogen does exist, becoming active in the presence of oxygen or of a weak acid—e. g. the endosperm juice gave, directly after trituration, for amylase 3 mg. CuO, and after twenty-two days of aseptic maceration 73·2 mg. The juice from scutella and embryos gave in the first test 4 mg., and in the last 80·3 mg. CuO.

One does not wish to say that all the cells of the Barley endosperm must be dead from the beginning of germination. Microscopic researches on vitality showed that if a residue of vitality does exist in the endosperm, we can find it only in the immediately sub-aleuronic tissue, because only there (with difficulty on account of the accumulation of reserve material) can we find a trace of plasmolysis, and because only there can we see, after appropriate staining, vestiges of nuclear substance, though we cannot speak of a well-defined nucleus.

Wheat.

In Wheat I was able to get complete emptying of isolated endosperms in free air. In the chloroform atmosphere evacuation could not proceed, and the whole endosperm became very hard, so that in this case chloroform seems to stop both the cytasic and amylasic actions. The behaviour of the individual cells towards plasmolytic agents and stains would demonstrate that they do not renew their vitality during germination. No trace of nuclei could be made out in the endosperm except in the aleurone cells. The solution of reserve material does not prove the vitality of this tissue, because I have been able to show, by the usual methods, that the resting seed contains pro-amylase which becomes active under the influence of oxygen and dilute acids. E. g. in the first amylase test only a trace of CuO was found, while after twenty days (at 18°C.) 123·92 mg. CuO were obtained for 5 c.c. endosperm juice. The scutellum and embryo juice gave in the first test only traces of CuO, and in the last 40·26 mg. CuO.

In these experiments no account had been taken of the small volume that the scutellum and embryo occupy in the seed, so that juices which had been made with the same amount of water or dilute glycerine were more concentrated in the case of the endosperms than of the scutella and embryo. Therefore one could not exactly estimate the ratio of increasing amylase between the two parts; inasmuch as, the endosperm extracts being

more concentrated, the decomposition products would very likely undergo a secondary synthesis, or at any rate the breaking-down process would be stopped.

Concentrated juices were made with the same proportion of water and $\frac{1}{10}$ hydrochloric acid. For a concentration of 2 per cent. at room temperature, the following results were obtained :—

Date.	*Endosperm juice.*		*Scutellum and embryo juice.*	
	Sugar in 5 c.c.	*Amylase of 5 c.c.*	*Sugar in 5 c.c.*	*Amylase of 5 c.c.*
	CuO mg.	CuO mg.	CuO mg.	CuO mg.
July 3	12·5	8	26	12
,, 7	27	13	76	34
,, 16	31	42	80	274

As we can see from this table, there is a greater amount and a larger increase of amylase in the juice from scutella and embryos than in the endosperm juice. This would lead one to suppose that the largest quantity of pro-amylase always occurs in the scutellum, and that digestion of reserve materials takes place by its action. Against this we must, however, place the fact that the whole embryo with the scutellum constitutes but a very small part of the seed (about one-eighth, $\frac{1}{8}$·2 in weight), while the results of this experiment have been calculated as if the scutellum, &c., weighed the same as the endosperm.

In reality, then, the amount of pro-enzyme which became active in the endosperm in comparison with that contained in the scutellum and embryo, is shown as follows :—

Date.	*Endosperm juice.*		*Scutellum and embryo juice.*	
	Sugar in 5 c.c.	*Amylase of 5 c.c.*	*Sugar in 5 c.c.*	*Amylase of 5 c.c.*
	CuO mg.	CuO mg.	CuO mg.	CuO mg.
July 3	104·50	65·6	26	12
,, 7	221·40	106·6	76	34
,, 14	254·2	344·4	80	274

So in the endosperm of the Wheat we find much more pro-enzyme than in the scutellum and embryo, and its action in the presence of free oxygen and dilute acid may itself cause the digestion of the endosperm food material.

Most interesting is the influence of chloroform, which not only inhibits starch hydrolysis in the intact endosperms, but also at first hinders the solution of the cell-walls. This would indicate that the cytase is produced in this endosperm by living cells. However, as the endosperm cells must be considered dead for the most part, for the reasons given above, there is

no choice but to admit that cytase production occurs only in the aleurone cells, or at most in the directly sub-aleuronic layers.

Wheat was used for a comparative study, by staining methods, of what happens in the amyliferous cells of the seed from the time of the beginning of endosperm formation up to the ripening of the seed.

Results were chiefly obtained by staining properly-fixed material with iodine green, methyl green, malachite green, gentian violet, and by double staining with methyl or malachite green and eosin, gentian violet and eosin, iodine green and orange. With single staining, both in the first stages of endosperm development and in more advanced stages (but always green), a sharp coloration of the nuclei in the starchy cells was obtained. These nuclei were easily distinguished among the starch grains, though the latter were present in great abundance in the later stages and had reached their proper size.

Eosin and orange gave a coloration of the cell protoplasm, while the nuclei absorbed their peculiar stains as iodine green, methyl green, malachite green, gentian violet, &c., in other stainings, and were clearly distinguishable in the middle of the cells, showing a perfectly normal structure.

As it might be thought that in sections of old endosperms which had been boiled in dilute acid in order to remove the starch, the nucleus would have lost its property of absorbing its peculiar stains on account of the treatment received, young cells with normal nuclei were treated in the same way. In spite of the treatment the nucleus and protoplasm of all the endosperm cells still retained to the full their property of absorbing stains. So it cannot be said that the removal of the reserve starch by dissolving out with dilute acid alters the chemical composition of the nuclear substance, even in ripe and germinating endosperms, sufficiently to hinder staining.

From all these observations it can be inferred that the cells of Wheat endosperms do not regain vitality during germination ; but the fact remains that they can empty themselves, even when cut off from the embryo, by means of the activation and functioning of their amylolytic enzymes, and that chloroform has a remarkable inhibiting action on this evacuation.

Rye.

With Rye (*Secale cereale*) a total evacuation of the endosperm was obtained by the same methods, with a copious diffusion of reducing sugars and other digestion products (albumins) in the surrounding liquid ; in the early stages of germination there occurred a complete disintegration of the starchy tissue by separation of the individual cells. Chloroform has no action upon the starch hydrolysis, nor on the dissolution of the walls, nor on the evacuation of the entire endosperm, for, though the seeds became a little

dark and hard at first, the usual separation of cells occurred, with a plentiful production of reducing sugars, which diffused in the external liquid. From this it may be inferred that the Rye endosperm is dead right up to the aleurone cells. As the hydrolysis of the walls precedes the starch hydrolysis, the latter cannot be ascribed to vital activity of the cells. While in Barley the hemicellulose is first dissolved, and then, almost immediately, the cellulose as well, here the pectic substance of the middle lamella first disappears, and only in a very advanced stage of evacuation, when the starch is almost entirely dissolved, does the hydrolysis and dissolution of the cellulose walls and hemicellulose thickenings begin.

As Puriewitsch does not in any way mention these dissolution processes of the cell-walls, which, moreover, he has also overlooked in Barley and Wheat, the question arose as to whether this Rye might behave differently from other kinds of Rye. I have to thank Professor Pirotta, who was able to procure for me several different kinds of Rye, from various Botanic Gardens, e. g. *Secale cereale* from the Botanic Gardens, Paris; *Secale cereale* from the Botanic Garden, Lyons; *Secale cereale* from Zuttich; *Secale cereale aestivum annuale* from the Seed Station, Zurich; *Secale cereale perenne* from Zurich; *Secale cereale* from the Botanic Gardens, Marburg; *Secale montanum* from Paris; *Secale montanum* from Lyons; *Secale cereale* from Utrecht, &c. In all these samples the same phenomena have been observed during endosperm evacuation as those mentioned above for the Roman Rye. Therefore there is no doubt that the Rye on which Puriewitsch worked must have shown the isolation of the endosperm cells.

The following experiments show that the question is really one of a cytolytic enzyme:—

A number of seeds of Rye were soaked for two days in water, and were then pounded and mixed with 25 c.c. water and 25 c.c. glycerine and thymol.

The paste was pressed through a cloth and a portion of the juice was boiled in order to kill the enzymes.

In both these juices boiled sections of Lupin cotyledons were placed, and the whole was set to digest at a temperature of 47°C.

After twelve days the sections in the unboiled juice showed the hemicellulose layers of the walls decidedly corroded, while they were entirely unaltered in the boiled juice.

In order to ascertain whether this enzyme proceeded from the scutellum or was contained in the endosperm cells themselves, two separate extracts of the endosperms and the rest of the seeds were made in the way above described. The usual boiled sections from the cotyledons of Lupin were put in these extracts and left to digest at 47°C. After nine days the hemicellulose thickenings were found strongly corroded in both cases.

Sections from Rye seeds were put into the same extracts, and after

two days wholly lost the amyliferous tissue, nothing remaining of the sections but the aleurone layer and seed envelopes.

Hence it may be concluded that in the Rye endosperm there is a cytase which is not confined to the scutellum. Certainly, it is true that the endosperms had stood for twenty-four hours in water before they were cut from the scutella and embryos, a time which might seem sufficient for exosmosis of enzyme from the scutellum; but it is easy to prove that if Rye endosperms are put to evacuate in the usual manner, from which the scutella and embryos have been cut before the seeds were moistened, the same phenomenon of rapid isolation of the cells may be observed.

Moreover, as direct experiments have proved, the Barley cytase also dissolves the cell-walls of Rye and Wheat very rapidly, while it has no action upon those of Maize. So Rye is not alone in producing an energetic cytase soon after the beginning of germination.

The disintegration of the amyliferous tissue at the beginning of evacuation shows in a convincing way that there is no trace of vitality. Nevertheless, the researches on cytological behaviour were repeated on the Rye. The results did but confirm the above conclusion. In the endosperm cells of Rye no trace of plasmolysis could be seen, and it was impossible by any means to discover a nucleus in the cells filled with starch.

By vital staining the whole amyliferous part of the endosperm stained immediately and completely.

In this case also self-evacuation is caused by a pro-enzyme which becomes active in presence of air or of dilute acid. For instance, an endosperm juice, under conditions of aseptic autolysis, gave in the first amylase test only a trace of CuO, while after twenty-one days 129·93 mg. were obtained for 5 c.c. juice; in the corresponding scutellum and embryo juice only vestiges were found in the first amylase test, while in the last 25·6 mg. CuO were obtained.

Conclusions.

The starchy endosperm of the investigated grains, Maize, Barley, Wheat, and Rye, can digest itself in the absence of the scutellum and other parts of the embryo, though to a very different degree; hence the diverse results reached by previous authors.

The self-emptying can go on in the absence of any vitality in the amyliferous cells, because the starch hydrolysis is accelerated by a strong amylase which, little by little, arises from a pro-enzyme which exists in the endosperm of the resting seed, and becomes active even though every trace of vitality has been removed from the entire endosperm or seed by mechanical means. Nevertheless, one cannot deny any vitality whatever to the endosperm cells. On the contrary, our researches lead one to admit that vitality, which is certainly possessed by the aleurone cells situated at

the periphery of the endosperm, is also retained in one or several sub-aleuronic layers, whence it lessens by degrees till it totally disappears towards the middle of the endosperm, as well as in the part near the scutellum.

This is clearly seen in the Maize, the endosperm of which shows well-marked, though strangely deformed, nuclei in the cortical, glutinous portion, while they are not to be brought in evidence in the farinaceous central portion which forms the bulk of the endosperm.

In Barley and Wheat, if any trace of vitality is conserved in the amyliferous cells, it must be sought in the immediately sub-aleuronic layers, the larger part of the endosperm being quite dead.

The rapid dismembering of the Rye endosperm at the very beginning shows that it is entirely dead. Its disintegration is due to the influence of a powerful cytase that precedes the amylase action, and dissolves the middle lamellas, while the cellulose layers of the walls are retained for a long time. The cells become isolated, but remain entire with intact starch in the endosperm cavity, so that one can no longer speak of an endosperm tissue.

A similar fact is to be noted in Wheat and Barley, but less marked and at a more advanced period of evacuation; but the phenomenon does not occur in Maize, though a cytase becomes active during the evacuation of this endosperm also.

The discrepancies of preceding authors are very likely due to the fact that they used different species of corn. So that Puriewitsch, Grüss, and Linz, who worked chiefly with Maize, are not wrong in describing its endosperm as partly living, while Brown and his co-workers are right in asserting that the endosperm of Barley is a dead magazine of reserve food.

Between the partly living endosperm of Maize and the totally dead endosperm of Rye, and also between the different parts of a grain of Maize or Wheat, one finds a gradual decrease in vitality. Nevertheless a rapid self-emptying of isolated grass endosperms can take place, for they contain pro-amylase, pro-cytase, &c., which still exist after the death of the cells, and yield large quantities of active enzymes after soaking in water at ordinary temperatures.

The amount of energy needed for the hydrolysis of starch and hemicellulose is very small, and can originate from the oxidasic, i. e. extra vital, respiration of the sugars formed. Herein one sees a difference between carbohydrate and oil reserves; in the latter only vital activity can supply the energy needed for the complicated formation of sugars, starch, &c., from the reserve fat. Indeed, I have shown in other papers ('07) that the endosperm of the Castor-oil plant (*Ricinus communis*) retains its vitality during the whole process of germination or artificial evacuation.

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Further Contributions to the Cytology of the Ascomycetes.

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With Plates XXVI and XXVII, and a Figure in the Text.

RECENT investigations by one of us (13) have shown that in *Humaria rutilans* a meiotic reduction, involving transverse fission of the heterotype chromosomes, takes place in the first two divisions in the ascus, and further, that it is followed, in the third mitosis, by a second, or brachy-meiotic reduction of a simpler type.

These processes accord with the observations of Harper (19) on *Phyllactinia* but have not been described in other forms; the present researches were undertaken as a first step towards ascertaining whether they are of general occurrence among Ascomycetes. We have found, in the two species with which this paper deals, intermediate stages between the early union of the chromosomes in *Phyllactinia* and their independence during the stages which precede reduction in *Humaria rutilans* and in higher plants and animals.

Material was obtained in Windsor Park during the autumns of 1906 and 1907, and was fixed in the field in various strengths of Flemming's fluid. Before staining, many of the slides were immersed for three or four hours in a solution of pepsin [1] and 0·2 per cent. hydrochloric acid at a temperature of 38° C. This treatment facilitated the study of the chromatin. Sections were cut 5–10 μ in thickness and were stained with Heidenhain's iron-haematoxylin or with Flemming's triple stain.

OTIDEA AURANTIA.

This species has a large, orange apothecium which increases considerably in size after the asci have begun to form. In the early stages of development a large cell with scanty contents is present, and represents,

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

[1] See Farmer and Digby (10), p. 165.

we believe, a disused ascogonium (Pl. XXVI, Fig. 1). The hypothecium is pseudo-parenchymatous, and consists of polygonal, closely placed cells; this arrangement differs from that in *H. rutilans* where the ramifications of individual hyphae can be distinctly traced. The hypothecial nuclei are too minute for satisfactory investigation, and consequently the attempt was early abandoned to observe the first and presumably pseudapogamous fusion.

In the sub-hymenial layer the ascogenous hyphae are distinguished by their dense contents. The nuclei are vacuolate, and, though larger than those of the hypothecium, they are still difficult to study. We found no indication of a conjugate arrangement.

In *Otidea aurantia*, Mass., as in *H. rutilans*, the first divisions in the ascus constitute a meiotic phase, and here also they correspond closely to the description given by Farmer and Moore (9). The various stages are almost diagrammatic in clearness (Figs. 2–10), and we feel no doubt as to their significance.

After the first contraction (Fig. 2) the spireme splits longitudinally (Fig. 3), synapsis takes place (Fig. 4), and four loops are formed (Fig. 5); in the limbs of these the longitudinal fission is seen, and they break apart to form the four gemini (Moore and Embleton (21)) or bivalent chromosomes (Fig. 6) which undergo considerable contraction. A transverse fission takes place on the heterotype spindle (Fig. 8), and the longitudinal fission is completed on that of the homotype (Figs. 11, 12). Four chromosomes thus travel to each pole both in the first (Fig. 9) and in the second mitosis (Figs. 12, 13).

During the early stages of meiosis the fusion in the ascus takes place.

The third division is inaugurated by a contraction[1] of the chromatin thread (Fig. 14) and two or sometimes four chromatin masses become visible (Figs. 15, 16). The spindle is then formed and in the early metaphase two long chromosomes are observed (Fig. 17). The exact sequence of the prophases is difficult to determine, but it seems clear that the two chromosomes of the metaphase are bivalent and that each represents two of those which pass to the poles in the previous mitosis. On the spindle they divide, and two small daughter-chromosomes travel to each pole.

This division appears, like the corresponding one in *H. rutilans*, to be brachymeiotic in character and to result in the separation of *different* portions of the nuclear thread.

During all three divisions in the ascus one or two granules are frequently seen within the nuclear area (Figs. 8, 13, 17). They may be connected with the centrosome by 'fibres' resembling those of the spindle

[1] Contraction is sometimes seen in the daughter nuclei after the first mitosis, the chromatin forming a dense mass at the end of the nucleus remote from the plane of division. This differs from the contraction of a chromatin *thread* observed in the third mitosis here and in both divisions in *P. vesiculosa*; it probably represents a stage of reconstruction comparable to Fig. 32.

(Figs. 11, 20), but they do not travel towards the poles (Fig. 10). Similar radiations are sometimes attached to the nucleolus (Fig. 18).

Spindle formation and the development of the spores take place as in *Peziza vesiculosa* and will be described in connexion with that species.

PEZIZA VESICULOSA.

Peziza vesiculosa, Bull. (*Pustularia vesiculosa*, Fckl.), has a conspicuous buff apothecium in which the phenomenon of 'puffing' is well shown. It has been studied by a number of observers; in it Dangeard (7) first saw the fusion in the ascus, and it has been more recently investigated by both Maire (20) and Guillermond (14).

The hypothecium is very similar in appearance to that of *Otidea aurantia* but an ascogonium was not recognized. Here also we were unable to observe a pseudapogamous fusion.

We can confirm Maire's statement that, before the bending over of the crozier, the nuclei in the ascogenous hyphae are not conjugately arranged, but we have been unable to recognize with certainty the single division which he describes as giving rise to the nuclei destined by a simultaneous mitosis to produce the nuclei of the ascus.

The divisions in the ascogenous hyphae are quite normal (Figs. 21, 22) and show about eight chromosomes on the equatorial plate (Fig. 21). Afterwards the subterminal cell of the hypha is cut off and gives rise to the ascus, as described by Dangeard. In the cases observed by us the fusion in the ascus (Fig. 23) took place at about the time of the first meiotic contraction.

The prophases of the first division are not as clearly defined as in *Otidea*; the regular occurrence, however, of contraction phases (Figs. 24, 26), as described also by Maire, sufficiently indicates the existence of the usual reduction at this stage.

Guillermond describes the formation of eight chromosomes, the spindle being at the same time developed in the centre of the nuclear area. In our material, however, spindle formation took place by the method recorded by Harper (15, 19) for *Erysiphe* and other species. The two centrosomes are first observed lying close together (Fig. 27) with a cone of radiations passing out from each. A little later, as they move apart, the radiations come into contact (Fig. 28) and a spindle is formed. It is at first a good deal bent, and is placed across the shorter axis of the nucleus (Fig. 28); as development proceeds it straightens out and usually comes to lie parallel to the longitudinal plane of the ascus (Figs. 29–31). The centrosome remains firmly attached to the nuclear membrane, so that the latter is often drawn inwards when the spindle is short (Fig. 29).

In the early stages of division eight chromosomes are visible on the

spindle (Figs. 28, 29) and, in the anaphase, eight travel to each pole (Fig. 30). We were not able to find four chromosomes, as described by Maire, in definitely uncut nuclei at any stage during this division.

On the spindle of the second or homotype division (Fig. 34), and also on the spindle of the third (Fig. 38), four chromosomes appear; they divide so that, in both cases, four pass to each daughter-nucleus (Figs. 35, 39, 40). Their formation is preceded by the withdrawal of the chromatin towards one side of the nucleus (Fig. 33); this may be regarded as analogous to one of the meiotic contractions and as indicating the moment of pairing of the eight chromosomes of the heterotype telophase. Since contraction takes place in *both* the second and the third prophase (Figs. 33, 37) it would seem that the union of the paired chromosomes is very slight and breaks down between the end of the second and the beginning of the third division; this conclusion is also borne out by Maire's and Guillermond's observations.

Guillermond has described eight chromosomes throughout the second and third divisions, and Maire eight protochromosomes in both prophases. In our material the number in the anaphases and early telophases of these divisions is clearly four, but it is not impossible that, in some cases, the eight chromatin bodies found on the spindle may represent the 'protochromosomes' of Maire rather than, as we have considered, the separated daughter-chromosomes.

In either case, by the end of the third division a second reduction has been accomplished and the eight chromosomes of the heterotype telophase have been replaced by four. The process by which this is accomplished appears to correspond to that in *H. rutilans* and *O. aurantia.*

Before the third division is complete spore formation begins. The spindle elongates, and during the telophases and the early stages of reconstruction of the daughter-nucleus a beak is formed (Figs. 43, 44) and appears to push actively towards the periphery. At the same time changes take place in the cytoplasm (Figs. 41–44); its staining capacity increases and the astral rays are bent backwards, giving the arrangement first described by Harper (15). The appearance of the rays suggests that currents of altered cytoplasm are flowing back around the advancing nuclear beak. A little later the upper portion of the spore is seen to be defined by a limiting membrane (Fig. 45), while the lower part is irregularly outlined by the sides of neighbouring vacuoles, and becomes rounded off at a later stage.

The arrangement of the vacuoles is in general very regular: a large one fills the lower part of the ascus and another occupies its apex. Seven others, more or less well-defined, are left by the first, second, and third mitoses; and the main body of cytoplasm is thus broken up into eight masses within which spore formation takes place (Fig. 44).

Irregularly shaped and multinucleate spores, and also asci which contained more than two nuclei at their formation, were several times observed.

The paraphyses are septate; in the earlier stages of development their nuclei are small and contain a single nucleolus, the rest of the stainable material being regularly distributed (Fig. 46). Later the nuclei become enlarged and vacuolate, and the nuclear contents are massed into deeply staining lumps (Fig. 47).

The Divisions in the Ascus.

The chromosomes have been counted in comparatively few Ascomycetes, a great part of our knowledge being due to the investigations of Maire (20) and of Guillermond (14). Harper, also, has studied several species and especially *Phyllactinia corylea* (19), where he found eight chromosomes throughout the life-history. This case is somewhat peculiar, as the chromosomes pair directly after the union of the nuclei, and thus their number remains unaltered while their valency is first doubled (in the oogonium) and later quadrupled (on the fusion in the ascus). In the same way reduction consists not in the diminution of the number of the chromosomes but in the halving of their valency.

In *Humaria rutilans* (13) the number of chromosomes in the mycelial nuclei before fertilization has not been counted; in the ascogenous hyphae (after the pseudapogamous fusion) there are sixteen; and the same number appears throughout the first and second divisions in the ascus and in the prophases of the third, when the meiotic reduction and asexual fusion are complete. In the third telophase there are only eight chromosomes and a second reduction—the brachymeiotic—has thus taken place.

In *Otidea aurantia* we were unable to count the chromosomes before the fusion in the ascus. The first two divisions in the ascus are, however, obviously meiotic, and the chromosomes are then four in number. In the third prophase *two* chromosomes appear, and these divide on the spindle so that two pass to each pole.

In *Peziza vesiculosa* the nuclei have been investigated by Maire (20) and by Guillermond (14), as well as by ourselves. Guillermond finds eight chromosomes throughout the divisions in the ascus. Maire also observes eight in all three prophases, but he regards these as protochromosomes and describes them as fusing to form four in the metaphase. In the late anaphase of the first division eight chromosomes reappear and are interpreted by him as representing the prematurely separated daughter-chromosomes of the homotype mitosis; in the second and third anaphases he finds only four.

We have observed eight chromosomes in the ascogenous hyphae, and also, like Guillermond, throughout the first division in the ascus. We regard eight, therefore, as double the postmeiotic number, produced either (in the ascogenous hyphae) by a pseudapogamous fusion alone, or (in the ascus) by an additional fusion combined with meiotic reduction.

In the second and third divisions we find four chromosomes throughout. It seems to us, therefore, that brachymeiosis takes place, as in *Humaria rutilans* and *Otidea aurantia*, in the third division in the ascus, but that, just as in *Otidea* the chromosomes unite in the third prophase, so here they are paired during the prophases of the previous division. If this union were somewhat deferred, Maire's eight chromatin masses would of course appear, fusing later to form four; and if, as in *Humaria rutilans*, it were altogether omitted the eight chromosomes of Guillermond would be observed throughout the second division and in the third prophase. It is to be regretted, however, that neither author figures all the stages he describes.

Maire (20) and Guillermond (14) have also investigated *Galactinia succosa*. Here they each find four chromosomes on the heterotype spindle preceded by a varying number of protochromosomes. In the early anaphase they find four chromosomes passing to each pole, but in the late anaphase eight are present. Eight also appear in the second prophase and unite on the spindle to form four. Four were observed in the second anaphase and throughout the third division.[1]

From the observations detailed above, the following points seem to us to emerge:—

(1) In *Humaria rutilans* there is no obvious mechanism for the separation of allelomorphs in brachymeiosis.

(2) In *Otidea aurantia* such a mechanism is apparent, the chromosomes uniting in pairs during the brachymeiotic prophase.

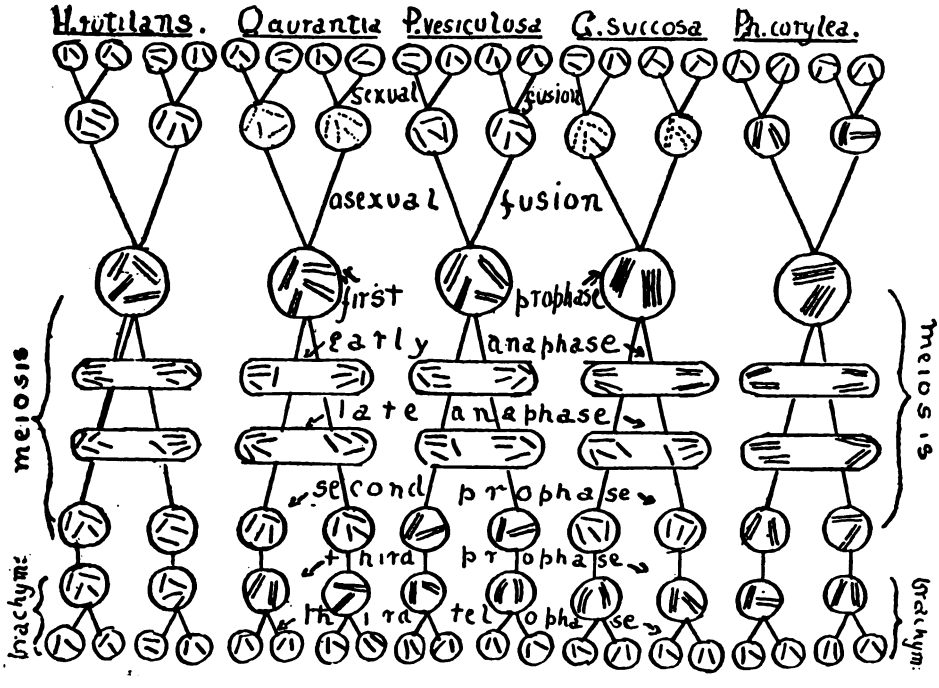
(3) In *Peziza vesiculosa* the time of the corresponding union seems to be variable; it took place in our material during the early stages of the division which precedes brachymeiosis.

(4) In *Galactinia succosa* pairing is brought about on the spindle of the first division, but it is very unstable, the four chromosomes separating into eight from time to time.

(5) In *Phyllactinia corylea* the chromosomes pair immediately after the nuclear union in the ascus, and the same is the case with regard to sexual fusion.

[1] Another case of great interest is that of *Morchella esculenta*, where Maire (20), after observing eight chromatin bodies in the prophase of the first division in the ascus, finds four in the metaphase and anaphase of the third, and also *in the division of the spore nuclei*. This, so far as we are aware, is the only instance in which the chromosomes have been counted between brachymeiosis and fertilization. Unfortunately the species has not been fully investigated.

We have represented these differences diagrammatically in the accompanying text-figure, in which, for the sake of uniformity, we have assumed the minimum number of chromosomes to be two throughout.



The Sexuality of the Ascomycetes.

In the development of a knowledge of these forms history has more than once repeated itself.

In 1791 Bulliard (4) described the asci as female organs and suggested that their fertilization was accomplished by the bursting of the paraphyses, which he regarded as male.

In and after 1863 the classical researches of De Bary (8) and his pupils established the existence of archicarps and antheridia in a number of species; they brought forward evidence of the occurrence of fertilization at this stage in some cases, and of a corresponding 'reduced' development in others. They regarded the ascus as a spore mother cell.

From 1872 onwards the extensive researches of Brefeld (3) appeared. He denied the sexual character of the organs observed by De Bary and attributed a vegetative significance to their fusions.

In 1894 Dangeard (7), investigating *Peziza vesiculosa* and some other forms, discovered the fusion in the ascus. He accepted the sexuality of

the Ascomycetes in a new sense, held the ascus to be an egg in which fertilization occurred, and regarded the archicarp and antheridium, when present, as merely vestigial.

In 1895 Harper (16) reinvestigated *Sphaerotheca humuli*; he was able to observe the fusion of a male and a female pro-nucleus in the oogonial cell of the archicarp, and further to confirm, for this species, Dangeard's statement that a nuclear fusion takes place in the ascus. The existence of two fusions in the life-history of the Ascomycetes was thus recognized and has since been confirmed, among normally sexual species, in *Erysiphe* (Harper (17)), *Pyronema* (Harper (18)), *Boudiera* (Claussen (5)), and *Phyllactinia* (Harper (19)), and among pseudapogamous forms in *Humaria granulata* (Blackman and Fraser (2)), *Ascobolus* (Welsford (22)), *Lachnea* (Fraser (12)), and *Humaria rutilans* (Fraser (13)). In all these fungi the two fusions have been actually described, while strong evidence of their occurrence has been brought forward in a number of others.

In 1907 Claussen (6), in a preliminary paper, re-described the development of *Pyronema confluens*. He stated that the nuclei pair, *without fusing*, in the ascogonium, that they travel in pairs, dividing conjugately, up the ascogenous hyphae, and eventually fuse in the ascus. Claussen concludes that a single fusion, that in the ascus, occurs, and that it represents the ultimate union of male and female pro-nuclei first associated in the ascogonium. He regards it as exactly comparable to the fusions in the teleutospore and basidium, and as forming, like these, the completion of a sexual act initiated at an earlier stage.

This may imply, as Claussen seems to think, a definite contraversion of the fusions observed by earlier workers and by himself (5), and his generalization is so far satisfactory that it brings the Ascomycetes into line with the Uredineae and other Basidiomycetes, and does away with the difficult question of the significance of the second, or so-called asexual, fusion.

It might perhaps be conceivable, in accordance with this view, that confusion should have arisen, in earlier work, with regard to the behaviour of the minute nuclei of the coenogamete, but it seems to us improbable that the sequence of stages in uninucleate forms, such as the mildews, should have been misinterpreted. In these species not only has the first fusion been observed, but a stage has been several times recorded (Harper (16), (17), (19), Blackman and Fraser (1)) when the antheridium is already empty and the oogonium contains a *single nucleus*; such a stage follows the entrance of the male nucleus into the oogonium and obviously implies fusion.

Claussen's views, moreover, as applied by him to Ascomycetes in general, are difficult to reconcile with the observations recorded both here and previously, as to the reduction phenomena in the ascus.

On the other hand, it has already been noticed that in pseudapogamous forms fusion may be delayed till the nuclei are just leaving the ascogonium, and that in *H. rutilans* it occurs in the ascogenous hyphae; it seems possible, then, that in *Pyronema confluens*, the fusion may sometimes be so much retarded as to actually take place in the ascus. There is, however, necessarily no proof that the large nuclei figured by Claussen in the ascogenous hyphae are not the result of fusion, even though they travel towards the hymenium in pairs, and though the later fusion stages were not seen.

In view of these various possibilities, a full account of the ascus divisions in *Pyronema confluens* would be of very great value and would no doubt solve the problem of nuclear fusion. It is quite to be expected that, in some Ascomycetes, one of the fusions should have been abandoned; and the conditions under which this may take place will be of considerable interest; it will also be of value to observe whether the method of reduction retained is the meiotic, which appears to represent the last stage of fertilization, or the brachymeiotic, which may be regarded as compensating an asexual fusion.

Sexual and Asexual Fusions.

In this connexion the distinction is of interest between asexual fusions and fusions which may be regarded as sexual in the widest sense. Fertilization, in all cases studied, is followed by a meiotic phase, the distinguishing characters of which appear to be (1) a contraction phase during which the chromatin filaments are temporarily massed together; (2) a longitudinal fission throughout the length of the spireme (this fission, though occasionally obscured, persists till the homotype metaphase, when it forms the line of separation of the daughter-chromosomes); (3) a second or synaptic contraction. Here the chromosomes become closely massed together, and, as the contraction loosens, their number, or that of the corresponding loops, may be counted.

Brachymeiosis is more variable; its most essential feature seems to be the formation of a given number of chromosomes, half of which pass to each daughter-nucleus. These chromosomes may be apparently independent or they may be paired at the beginning of the brachymeiotic division as in *Otidea aurantia*, or at an earlier stage as in *Peziza vesiculosa* and *Phyllactinia*; such pairing, in the two former cases, is associated with a contraction phase.[1] It seems, therefore, that some mechanism exists for the orderly distribution of allelomorphs.

[1] I have re-examined part of the material of *Humaria rutilans* from this point of view, but without finding a contraction either in the second or the third prophase. Such a result is quite to be expected, as there is no obvious pairing of the chromosomes in this species.—H. C. I. F.

Apart from the premature longitudinal fission of the heterotype, and the consequent occurrence of the homotype division, the difference between the two methods of reduction seems to lie in the extra contraction of meiosis. In all probability this contraction is the second, since Moore and Embleton (21) have observed the formation of the gemini in the cockroach before a spireme appears, and Harper (19) has found synapsis in *Phyllactinia* where the chromosomes are already paired. These facts suggest that the first meiotic contraction rather than the second is connected with the union of the chromosomes.

It seems, then, that meiosis is distinguished from brachymeiosis by its synaptic phase, and it seems not unlikely that this indicates the moment of some interchange of material between the already paired allelomorphs. If this be the case, the opportunity for interchange must vary considerably according to the extent of the synaptic contraction ; it would be of interest to ascertain whether there is any relation between this difference and the occurrence of mutation.

We are inclined to believe that brachymeiosis, since it lacks a second contraction, admits of less variation in its products than meiosis, and implies either the separation of the *entire* nuclei which fused, or at any rate a sorting of *unaltered* chromosomes. Similarly it might be possible to regard asexual fusion as essentially a temporary expedient, the result of such casual conditions as proximity (as when two nuclei associated in the same cell fuse); and sexual fusion, even when reduced, as a more permanent and significant process implying an interchange of parental material.

We are not prepared to suggest that the forms of reduction are never interchangeable ; but, while its phylogenetic history is perhaps the ultimate test of the nature of a fusion, it is noteworthy that there is no case known of a sexual process, however simplified, followed by any but a meiotic reduction.

Spore-formation.

Spore-formation in the Ascomycetes was first studied by Harper (15) in 1895, and his account has been several times confirmed. He concludes that the spore is bounded by the fibres of the polar aster, which bend round and fuse laterally to form a membrane.

Faull (11), in 1905, described the spore as cut out by the gradual differentiation, from the centrosome downwards, of a limiting layer of 'hyaline or finely granular' cytoplasm. This account, unlike Harper's, has been correlated with the processes observed in certain Phycomycetes.

In 1908 it was suggested by one of us (13) that the spore is in fact delimited by the astral rays, but that these represent currents flowing

out from the centrosome. Our present studies, especially on *Peziza vesiculosa*, have strengthened this point of view. It has been suggested that the centrosome is the seat of fermentive activities. On this hypothesis the centrosome, as it pushes outwards through the cytoplasm at the end of the third division, might be regarded as constantly generating a ferment. This ferment would flow back in its wake and would delimit the spore by producing a chemical change in the area through which it was distributed. It would ordinarily tend, as in the polar aster, to flow out equally all round the centrosome, but in this case, owing to the movement of its source, it would flow especially backwards. Its effect would be limited in certain directions by the occurrence of vacuoles and by the presence of the ascus wall. Whether the changes which thus take place are due to enzyme activity or to some other agent, we conclude that while the spore is to some extent bounded by neighbouring vacuoles, the main factor in its delimitation is an alteration of the cytoplasm, originating at the centrosome, and essentially similar in character to that which produces the aster.

Summary.

1. In *Otidea aurantia* traces of a probably functionless ascogonium are present; no such structure was found in *Peziza vesiculosa*.

2. In both species the first and second divisions in the ascus constitute a meiotic phase; this was investigated in some detail in *Otidea aurantia*, and was found to correspond closely to the description given by Farmer and Moore, the first division being diaschistic.

3. Fusion in the ascus occurs at about the time of the first meiotic contraction.

4. A second reduction takes place in the third division in the ascus. In *Otidea aurantia* the chromosomes pair in the prophases of this division; in *Peziza vesiculosa* they unite at an earlier stage.

5. We regard the presence of both meiosis and brachymeiosis in these forms, as well as in those previously described, as additional evidence of the occurrence of two fusions in the life-history of Ascomycetes.

6. The number of chromosomes in the first division in the ascus is four in *Otidea aurantia* and eight in *Peziza vesiculosa*; after brachymeiosis is complete there are two chromosomes in *Otidea*, four in *Peziza*.

7. The spores are delimited to some extent by vacuoles, but mainly by the astral rays. It is suggested that these may represent the paths of activity of an enzyme generated at the centrosome and producing chemical changes in the surrounding cytoplasm.

List of Papers.

1. Blackman, V. H., and Fraser, H. C. I. ('05): Fertilization in *Sphaerotheca*. Ann. Bot., xix, h. 567.
2. ——————————— ('06): On the Sexuality and Development of the Ascocarp in *Humaria granulata*. Quel. Proc. Roy. Soc., London, B. lxxvii, p. 354.
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18. ——————— ('00): Sexual Reproduction in *Pyronema confluens* and the morphology of the Ascocarp. Ann. Bot. xiv, p. 321.
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EXPLANATION OF PLATES XXVI AND XXVII.

Illustrating Dr. Fraser and Miss Welsford's paper on the Cytology of the *Ascomycetes*.

Figs. 1–20, *Otidea aurantia*.

Fig. 1. Section through ascogonium. × 950.

FIRST DIVISION.

Fig. 2. Two nuclei in the ascus, showing first meiotic contraction. × 2800.

Fig. 3. Fusion nucleus with longitudinally split spireme. × 2800.

Fig. 4. Synaptic contraction. × 2800.

Fig. 5. Nucleus passing out of synapsis; four loops are apparent and show longitudinal fission. × 2800.

Fig. 6. Chromosomes; longitudinal fission visible in the limbs. × 2800.
Fig. 7. Heterotype metaphase. × 2800.
Fig. 8. The same; later stage. × 2800.
Fig. 9. Anaphase. × 2800.
Fig. 10. Telophase. × 2800.

SECOND DIVISION.

Fig. 11. Homotype metaphase. × 2800.
Fig. 12. Anaphase; lower spindle cut obliquely. × 2800.
Fig. 13. Telophase. × 2800.

THIRD DIVISION.

Fig. 14. Brachymeiotic contraction. × 2800.
Fig. 15. Later prophase. × 2800.
Fig. 16. Later prophase. × 2800.
Fig. 17. Metaphase. × 2800.
Fig. 18. Early anaphase, showing radiations passing out towards nucleolus. × 2800.
Fig. 19. Anaphase. × 2800.
Fig. 20. Telophase. × 2800.

Figs. 21–47, *Peziza vesiculosa.*

Fig. 21. Equatorial plate in ascogenous hypha; formation of crozier. × 2600.
Fig. 22. Telophase in ascogenous hypha; the plane of section is probably at right angles to the crozier. × 2600.
Fig. 23. Nuclear fusion in ascus. × 2600.

FIRST DIVISION.

Fig. 24. First contraction of the chromatin. × 2600.
Fig. 25. Longitudinal fission of spireme. × 2600.
Fig. 26. Synaptic stage. × 2600.
Fig. 27. Early stage of spindle formation; heterotype chromosomes formed; part of the nucleus has been cut away. × 2600.
Fig. 28. Spindle lying across shorter axis of nucleus; eight chromosomes present. × 2600.
Fig. 29. Early metaphase. × 2600.
Fig. 30. Anaphase. × 2600.
Fig. 31. Telophase. × 2600.
Fig. 32. Reconstruction of daughter-nuclei. × 2600.

SECOND DIVISION.

Fig. 33. Contraction of chromatin. × 2600.
Fig. 34. Metaphase, showing four chromosomes. × 2600.
Fig. 35. Anaphase. × 2600.
Fig. 36. Telophase. × 2600.

THIRD DIVISION AND SPORE FORMATION.

Fig. 37. Contraction of chromatin. × 2600.
Fig. 38. Metaphase, showing four chromosomes. × 2600.
Fig. 39. Anaphase. × 2600.
Fig. 40. Telophase; lower nucleus in polar view. × 2600.
Fig. 41. Slightly later telophase; astral rays increasing in prominence. × 2600.
Fig. 42. Later stage, beginning of delimitation of spore. × 2600.
Fig. 43. Spore formation; appearance of nuclear beak. × 2600.
Fig. 44. Ascus, showing arrangement of vacuoles; reconstruction of nuclei almost complete; spore formation further advanced. × 2600.
Fig. 45. Upper part of spore clearly delimited; lower part not yet defined. × 2600.
Fig. 46. Young paraphyses. × 2600.
Fig. 47. Older paraphysis, showing vacuolate and disintegrating nuclei. × 2600.

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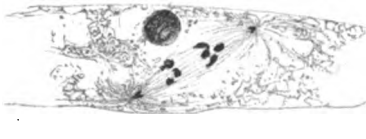

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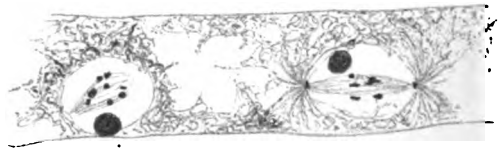

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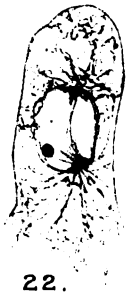

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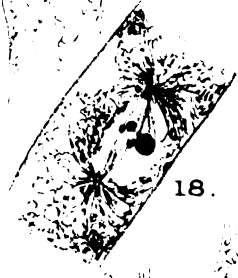

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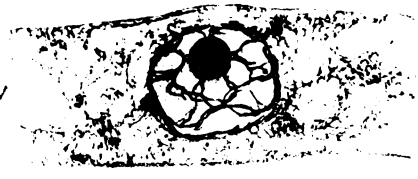

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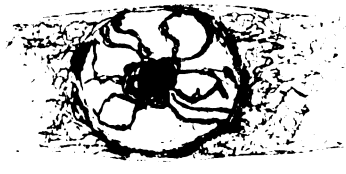

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OLOGY OF THE ASCOMYCETES.

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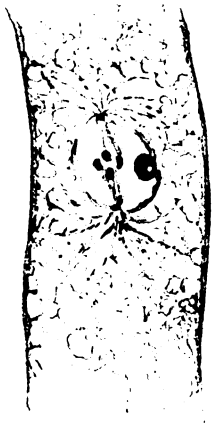

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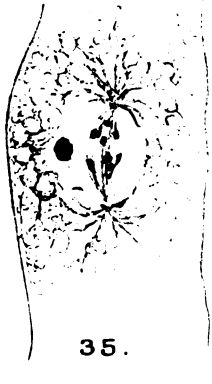

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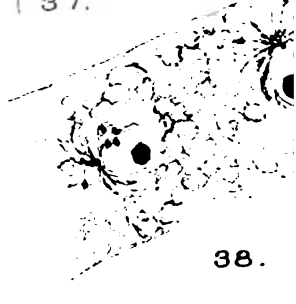

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LOGY OF THE ASCOMYCETES.

Observations on the Biology of Botrytis cinerea.

BY

F. T. BROOKS, B.A.,

Frank Smart Student, Caius College, Cambridge. Late Scholar, Emmanuel College.

With four Figures in the Text.

THOUGH the connexion between *Botrytis cinerea* and *Sclerotinia Fuckeliana* had been long surmised, no definite proof of the identity of these two forms was forthcoming until the recent appearance of a paper by Gy. de Istvánffi, entitled 'Études microbiologiques et mycologiques sur le rot gris de la vigne—*Botrytis cinerea* ou *Sclerotinia Fuckeliana*.' Istvánffi has come to the conclusion that *Botrytis cinerea* is indubitably the conidial stage of *Sclerotinia Fuckeliana*. This fungus causes a virulent disease of the fruit and foliage of the vine on the Continent. The diseased areas at first produce abundant conidiophores of the *Botrytis* type and sclerotia are formed later. Istvánffi has shown that these sclerotia, upon germination, produce either conidiophores of the *Botrytis* type or apothecia such as have long been known to characterize *Sclerotinia Fuckeliana*. In this country, *Botrytis cinerea* is generally found growing saprophytically upon the dead leaves and flowers of many plants. After being nourished in this way the mycelium has frequently the power of destroying living tissues by the secretion of a poisonous substance.

The group of fungi to which *Botrytis cinerea* belongs has been of particular interest to the plant pathologist ever since De Bary published his researches entitled 'Ueber einige Sclerotinien und Sclerotinienkrankheiten.' In that series of papers he dealt mainly with the life-history of *Sclerotinia Sclerotiorum*, especially in so far as it was the cause of a disease of the roots of *Beta*, *Raphanus*, and *Daucus*, and the foliage of *Phaseolus* and *Petunia*. This fungus has no conidial fructification, but produces abundant sclerotia, which germinate after a period of rest and give rise to apothecia. De Bary found that the ascospores were unable to cause direct infection of the living host-plant; if, however, the ascospores were sown upon some saprophytic medium, the young mycelium produced, upon

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

germination, possessed the power of destroying living tissues. He concluded that this power lay in the secretion of some poisonous substance or substances by the fungal hyphae, the tissues thus disorganized serving as food material for the further development of the fungus. In regard to the disintegration of the tissues, De Bary states that two phenomena are to be distinguished, viz. the death of the protoplasmic bodies and the destruction of the cell-walls. He came to the conclusion that an enzyme is the specific agent of the latter, while the death of the protoplasmic bodies might be due either to an enzyme or to an acid, or to both working together.

Marshall Ward, in his work on 'A Lily Disease', found that a fungus nearly related to *Botrytis cinerea*, or possibly identical with it, attacked plants of *Lilium candidum* in a very similar manner to that of *Sclerotinia Sclerotiorum* mentioned above. He attributed the disorganization of the tissues of the Lily to a cellulose-dissolving enzyme secreted by the fungus. He found that the conidia could bring about direct infection of the leaves and flower buds, though he noticed that the attack was stronger if some saprophytic nourishment was at hand to invigorate the young germ-tubes. R. E. Smith, in a recent paper, puts forward the view that some such substance as oxalic acid is secreted by the fungus and is responsible for the primary destruction of the tissues.

Kissling, in 1889, published an account of a disease of *Gentiana lutea* caused by *Botrytis cinerea*. He found that the conidia could not bring about direct infection of the leaves, though the young germ-tubes readily attacked the stigmas and anthers of the flowers. Doubtless this was on account of the lack of cuticularization of these parts. It is noteworthy that the species of *Sclerotinia* which cause the 'mummy' fruits of various kinds of *Vaccinium* infect their hosts in the same way.

In 1899 Nordhausen investigated further the question as to whether *Botrytis* conidia can infect living tissues. He found that non-cuticularized organs such as the anthers and petals of the Tulip and Crocus, and Moss leaves succumbed readily to the attacks of the germinating conidia. Only under such a condition as the following, however, did it result that leaves could be attacked. He placed conidia on the leaves of *Tradescantia* kept in a damp chamber, and regulated the deposition of dew. When the amount of dew deposited was small, the spores caused infection. He explained this, and the non-infection when the deposition of dew was greater, by supposing that in the latter case the poisonous substance secreted by the germ-tubes was diluted beyond the minimum intensity necessary to cause infection. Nordhausen mentions a number of factors in regard to the disposition of the host which might render it liable to destruction by *Botrytis*. He suggests that plants which have become etiolated through being kept in darkness would succumb the more readily to the fungus; also that dying leaves of ordinary plants would offer less

resistance to attack. However, he gives no details of infection under these conditions

In view of the uncertainty attaching to the power of infection of *Botrytis* conidia, the late Professor Marshall Ward suggested to me the advisability of investigating the matter again. At the outset of the work the growth of the fungus upon different media was tried. The results of this may be summarized as follows:—Upon grape extract stiffened with gelatine the fungus grew luxuriantly and produced abundant conidiophores of the normal type. Later, the structures known as 'organs of attachment' appeared in great abundance as brown specks, and occasionally sclerotia were produced.

In Klebs's solution, similarly prepared, the growth was sparse, only a few conidiophores of the normal type being produced. 'Organs of attachment' were rarely produced, and sclerotia were not observed. It was noticed that in succeeding cultures upon this medium the growth became somewhat more luxuriant. This points to the possibility of the fungus adapting itself in the course of time to a medium which was not originally favourable to its growth.

Upon bouillon with 10 per cent. gelatine the growth was comparatively feeble, and during the first three generations only conidiophores bearing microconidia were borne. This is additional evidence of the well-known plasticity of *Botrytis* upon culture media. The microconidial form of fructification has been recorded and figured by several observers, notably by Istvánffi, who obtained it upon glycerine cultures. In the fourth generation of the fungus upon the bouillon medium the growth was more luxuriant and the normal conidiophores were produced. It is not known whether this change in the mode of reproduction was dependent upon some alteration in the conditions of experiment, such as accounts for the different modes of reproduction in some Algae, as Klebs has shown. If this were the case, the alteration must have been a slight one, for the cultures were kept in a room where the obvious physical factors controlling the growth were the same. It may be that the fungus, having become accustomed during three previous generations to the bouillon medium, was sufficiently invigorated to produce again the normal type of conidiophore. No sclerotia and but few 'organs of attachment' were formed in the bouillon medium.

For the infection experiments Lettuce plants were almost exclusively used, as these are extremely susceptible to the attacks of *Botrytis*. When growing upon these plants the fungus produces abundant conidiophores of the normal type and, later on, sclerotia. At first, attempts were made to see whether the spores could directly infect the ordinary leaves. Thus spores from the grape extract medium were placed upon the leaves of normal plants kept uncovered in a greenhouse. In another series of ex-

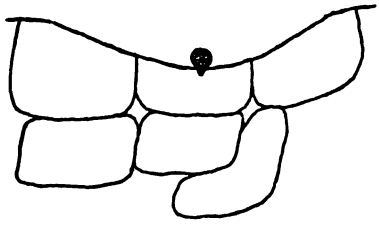
periments the spores were sown in drops of water upon the leaves. In no case, however, was infection brought about. Under the microscope it was seen that some of the spores had germinated. The short germ-tubes so produced soon collapsed. The explanation of this may be that the small amount of poisonous substance secreted at this young stage is insufficient to destroy normal tissues. A fuller discussion of this point will be gone into later in the paper. The same experiments were tried with plants which had been kept some days under a bell-jar and had therefore been living in a continually moist atmosphere. It might be expected that the tissues of such plants would be more easily penetrated by the secretions of the germ-tubes, but no case of infection was observed.

Kissling recorded the fact that the mycelium of *Botrytis* caused infection of a certain host the more speedily, the greater the number of 'generations' the fungus had lived on that host since its origin from a sclerotium. This suggested the possibility that spores derived from a mycelium which had lived a long time upon Lettuces might perchance be capable of infecting normal Lettuce leaves where spores derived from the grape extract cultures were impotent. Experiments were repeatedly tried with this end in view, but in no case was infection brought about. On the other hand, infection was in all cases induced by placing young mycelia in drops of the grape extract medium upon the healthy leaves. The young fungus invigorated in this way became the victor in its struggle with the green plant.

The effect of wounding normal plants was next tried. Leaves still attached to the plants were torn in various ways, and spores placed upon the wounded parts. A hot platinum needle was applied to certain of the leaves and spores placed upon the burnt areas. In every case infection ensued. Of course in such cases the juices which exude from the wounded areas provide saprophytic nourishment for the further development of the germ-tubes.

During these attempts to bring about infection by means of the spores, it was noticed that certain of the plants, several days after being experimented with, showed the well-known disease areas of *Botrytis* upon the lower leaves. It was observed that in all cases the leaves affected were becoming yellow. When the diseased area was small it could be seen that infection began at that part of the leaf upon which the spores had been originally placed. The plants which bore these infected areas on the yellowing leaves had been kept under bell-jars in bright light. It seemed fair to conclude from this observation that the spores were able to infect yellowing leaves although unable to infect those of normal green colour. This conclusion was tested repeatedly, and as often confirmed. It was apparent from the tests made that *Botrytis* conidia could bring about infection of leaves which had only just begun to turn yellow. To

ascertain how soon after the inoculation with spores, of the leaf just beginning to turn yellow, the penetration of the germ-tubes began, the following method was adopted:—Leaves just beginning to turn yellow were inoculated with spores, and from day to day the areas of the leaves on which the spores had been placed, were cut out and fixed in Flemming's weaker solution, and afterwards microtomed and stained. Upon examination of the sections it was found that the germ-tubes had already begun to penetrate the tissues the first day after inoculation. The germ-tubes pierce the cuticle and soon ramify in the cells which they have destroyed, as will be seen in the accompanying figures. It will be observed in Fig. 3 that the nuclei of the developing hyphae are extremely numerous, but very small. In Fig. 4 the nuclei are seen under a greater magnification.



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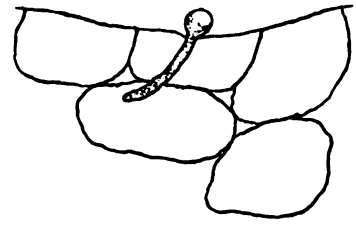

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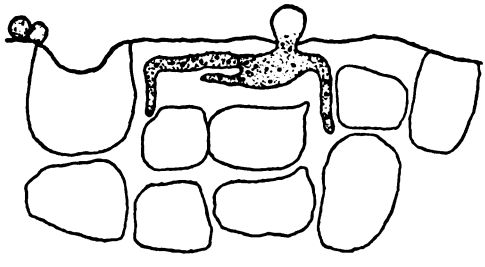

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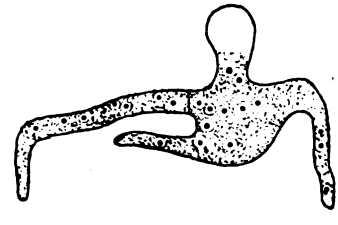

Fig 4.

These yellowing leaves must be considered to be in the incipient stage of decay, for they were never observed to recover their normal colour. They are by no means 'dead', however, if the term 'death' be applied to the state when the cells cease to be plasmolysable. These yellowing leaves live several days before they become flaccid and collapse. It would

be interesting to know what is the exact physiological condition of these yellowing leaves. That the power of carbon assimilation is dwindling rapidly seems certain, for under the microscope the colour of the chlorophyll granules is seen to be fading. One naturally inquires what is the cause of the direct infection of the yellowing leaves and the non-infection of the normal green leaves.

It may be that some chemotropic substance present in the cells of the yellowing leaf attracts the germ-tubes, whereas no such chemotropic influence is exerted by the normal leaf. Recently, however, Fulton has thrown considerable doubt upon the positive chemotropism of fungal hyphae.

Or is it possible that in the case of non-infection of a normal leaf, some substance from the epidermal cells diffuses through the cuticle in sufficient quantity to neutralize the effect of the small amount of poisonous substance secreted by the germ-tubes, whereas no such substance diffuses from the yellowing leaf? This would mean that something in the nature of an antitoxin accounts for the immunity of the normal leaf from infection by the spores. A third explanation is open to suggestion—it may be that the change in the vital activities of the leaf consequent upon yellowing induces some alteration in the composition of the external cell-walls, which enables the germ-tubes to penetrate them.

Subsequent research will show whether either of these explanations is valid.

Similar experiments to those outlined above were tried with plants which had been placed in darkness. At the time of placing the plants in the dark-room, all leaves which showed any trace of yellowing were cut off. After the plants had been kept in darkness five days, spores were placed upon the leaves, which had by that time begun to show signs of yellowing. The areas upon which the spores had been placed were cut out and fixed as described before. Upon microtoming and staining it was found that the germ-tubes had penetrated the tissues a day after inoculation. Three days after placing the spores upon the leaves large disease areas could be seen with the naked eye. In the same way, green leaves cut from healthy plants and placed under damp conditions in darkness, after being inoculated with spores, showed the characteristic disease areas immediately after they had begun to turn yellow. F. F. Blackman has shown in his experiments upon the respiration of starved Cherry Laurel leaves, i. e. leaves cut off the plant and placed in darkness, that soon after the period of maximum CO_2 production prior to death, the common mould fungus *Penicillium* begins to flourish upon the leaves. At this juncture the juices of the leaf-cells are escaping, and provide the necessary saprophytic nourishment for the spores of the moulds which are lurking on the surface. It seems from the experiments described above, that the time when *Botrytis* conidia cause infection

of the starved leaves is earlier than the time when such a truly saprophytic mould as *Penicillium* begins to flourish upon them. This is in accordance with the view which considers *Botrytis* to be a hemi-saprophyte. Its mode of nutrition cannot be placed in the same category of saprophytism with that of such a fungus as *Penicillium* or *Eurotium*; nor can it be considered a true parasite, for it kills tissues in advance of its own growth.

While the experiments already described were being carried on, another series was in progress, in which the Lettuce plants were grown under different conditions of mineral starvation. It was desired to ascertain whether plants, grown in a substratum which was deprived of certain mineral supplies, were more liable to infection by *Botrytis* conidia than plants grown in ordinary soil. Lettuce seeds were sown in fine sand, chemically pure, placed in circular glass jars. At the bottom of each jar a small inverted flower-pot had been placed in an inverted position, and passing through the hole in the flower-pot was a glass tube, as will be seen in the accompanying sketch. Some pieces of broken glass put around the flower-pot served to prevent the sand from falling and filling the air space within the flower-pot. Each day during the progress of the cultures the air was sucked up from the bottom of the jars by means of the glass tube. In this way the proper aeration of the roots was provided for. The precaution was taken of sterilizing each part of the apparatus separately, and each jar when fully set up was sterilized again prior to the sowing of the seeds. Black paper was bound round the outside of the jars in order to prevent the growth of Algae within.

The culture solutions chosen were those devoid of potassium, magnesium, nitrogen, and phosphorus, respectively, and of course there was a control experiment in which the solution used contained all the normal mineral ingredients. All the solutions were made up according to the formulae given on p. 224 of Macdougal's Textbook of Physiology. After the seeds had been sown the cultures were moistened with distilled water until the cotyledons appeared. Then the young plants were watered every other day for a fortnight with the culture solutions, after which they were treated in the same way every third day. On other days the sand was moistened with distilled water, if this was thought necessary. Care was taken to avoid any collection of liquid at the bottom of the jars, so there was no danger of the roots being water-logged. The cultures were placed under large bell-jars in a greenhouse, the temperature of which varied between 10° and 20° C. The plants grew well under the conditions of the experiment. Six weeks after sowing the seed each plant grown in the jar containing the normal mineral supplies, and in those devoid of nitrogen and magnesium respectively, possessed 4–5 ordinary foliage leaves, the largest being 1·5 c.m. in diameter. The plants grown in sand devoid of phosphorus were about half the size of those just mentioned, while those

deprived of potassium were intermediate in size between those described in the first category and the latter. In all cases, however, the plants appeared to be quite vigorous. One might suppose that after six weeks' active growth the influence of the reserve materials stored up in such seeds as those of the Lettuce would be greatly minimized ; and that the absence from the soil of certain of the normal mineral constituents would be felt throughout the regions where metabolism was in progress. At this stage *Botrytis* spores derived from diseased Lettuces were placed directly upon the leaves of the different cultures, the precaution having been taken of cutting off any yellowing leaves present. In none of the plants, however, did any sign of infection ensue. The inoculations were repeated, the spores being placed this time in drops of water upon the leaves. There was the same negative result as before. The whole series of cultures was repeated twice, but in no case did any evidence come to hand that the deprivation of certain mineral substances from the soil would induce the plants grown in it to succumb to direct infection by the spores.

It is clear, therefore, that, even by a great reduction in the amount of certain mineral constituents available, no such change can be induced in the constitution of the green plant as would account for the penetration of the germ-tubes of *Botrytis*. One supposes that the amount of food material stored up in the seed, together with the water and mineral substances absorbed from the soil and the carbon fixed from the air, enables the ordinary plant machine to elaborate a certain amount of protoplasm. If, e. g. a certain mineral substance is deficient in quantity, a less amount of protoplasm will be formed, but the protoplasm produced under these conditions will be of the same 'quality'—if one can use such a term—as that produced under conditions of normal nutrition. And if the protoplasm of plants grown under conditions of mineral starvation is of the same 'quality' as that of plants grown under normal conditions, there is no likelihood that the constitution of the former plants will be such as to lead to infection by the germ-tubes of *Botrytis*.

The results here obtained may be compared with those of Marshall Ward in regard to the parasitism of *Puccinia dispersa* upon species of Brome grasses grown under conditions of mineral starvation. He found that starvation of the host had no appreciable effect upon the ability of this fungus to infect. Of course the method of infection of *Botrytis cinerea* is totally different from that of *Puccinia dispersa*, but the aim of the culture experiments was the same in each case. With *Botrytis*, whose conidia are unable to infect the leaves of the normal Lettuce plant, and with *Puccinia dispersa*, whose uredospores readily infect the Brome grass, the influence of mineral starvation of the host upon the inability or ability to cause infection, respectively, was nil. The work on *Botrytis* outlined here confirms Marshall Ward's view that 'whatever may be the

causes at work in the living cell which confer immunity or predisposition on the species of host-plant, or which confer virulence or impotence on the spore, they lie deeper than nutrition.'

In conclusion, I would like to tender my hearty thanks for the help I have received in this work from the late Professor Marshall Ward and from Mr. F. F. Blackman and Mr. R. H. Biffen.

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Studies on the Evolution of the Angiosperms.

The Relationship of the Angiosperms to the Gnetales.

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With three Figures in the Text.

IN a former paper[1] we, conjointly, gave a sketch of the main conclusions respecting the ancestry of the Angiosperms, at which we had arrived after some years' study of the subject. The kind reception[2] extended to our endeavours to construct a working hypothesis, however imperfect, towards the solution of the problem, has encouraged us to pursue the matter further. We thus propose to elaborate certain questions which, in our preliminary paper, were purposely passed over or only treated summarily. These further studies we hope to publish from time to time, either conjointly or separately, in a series of which the present forms the first instalment.

PART I. THE RELATIONSHIP OF THE ANGIOSPERMS TO THE GNETALES.

INTRODUCTION.

In our general paper 'On the Origin of Angiosperms', the Gnetales were barely considered for reasons which we there stated as follows:—'We may conclude that the study of the Gnetaceae does not, and does not seem likely to, help us in understanding the phylogeny of existing Angiosperms. It would appear more probable that a knowledge of the descent of the latter,

[1] Arber and Parkin ('07). [2] Sargant ('08); Bessey ('07).

[Annals of Botany, Vol. XXII. No. LXXXVII. July, 1908.]

obtained from other sources, will itself shed light on the relationships of the former.'[1]

To this conclusion we still adhere, so far as the morphology of the fructifications of the Gnetales is concerned. But we propose to consider here whether the Strobilus Theory of Angiospermous descent, as outlined in our previous paper, will not help towards a clearer insight into the real relationships existing between the two groups, and thus permit us to find a place for the Gnetales in the table of phylogenetic relationships which is given at the conclusion of that paper. It will be found that we, after much consideration, have arrived by this means at an hypothesis which appears to us to remove, for the time being at any rate, the great uncertainty which has hitherto existed in regard to the closeness of these relationships.

It has been a vexed question for many years past whether the Gnetales themselves did not give rise to the Angiosperms. Previous conclusions on this point will be fully reviewed in the next section of this paper. We may, however, illustrate here the diversity of opinion which has existed by means of two quotations, between which a period of sixteen years elapsed. On the one hand, in 1885, the French Palaeobotanist, Renault,[2] who had paid considerable attention to certain Palaeozoic fructifications (*Gnetopsis*) then believed to belong to the Gnetales, decided that 'Les Gnétacées établissent une transition entre les Gymnospermes et les Angiospermes'. On the other hand, in Coulter and Chamberlain's[3] authoritative summary of the Gymnosperms, published in 1901, we find the following statement with regard to the Gnetales: 'Certain angiospermous characters which they display have suggested that they may have given rise to Angiosperms, but such a theory seems to have been abandoned by most morphologists.'

We are well aware that the problem presents great difficulties. There are but three genera in existence, two of which are very highly specialized in relation to their environment, and the fructifications of all three show comparatively little variety. Consequently few clues are afforded respecting the possible homologies of their individual organs. When, in addition, it is admitted that the fossil record of the group is really *nil*, it is obvious that the data at our disposal are of the slightest. It will therefore be to the credit of what we have elsewhere termed the Strobilus Theory,[4] if, by its aid, we can evolve a reasonable hypothesis of the relationships of the Angiosperms to the Gnetales. It may also be remarked that the two theories, the Strobilus Theory of Angiospermous descent, and the parallel and similar theory about to be elaborated here respecting the Gnetales, stand or fall together.

Although, so far as we can determine, there is at present no evidence of the Gnetales in the fossil state, we are not thereby precluded from an

[1] Arber and Parkin ('07), p. 34.
[2] Renault ('85), vol. iv, p. 171.
[3] Coulter and Chamberlain ('01), p. 112.
[4] Arber and Parkin ('07), p. 36.

attempt to restore in imagination the essential features of the fructification of the immediate ancestors of the group. There appear to us to be three possible alternatives from which to choose. These may be stated as follows:—

(1) The Gnetales were in past times, especially during the Tertiary period, a great and complex group, forming one of the dominant factors in the vegetation of the period. On this supposition the three surviving genera are probably very aberrant members.

(2) The Gnetales are quite a new group, with little or no past history.

(3) The Gnetales were in existence in Tertiary times, but have always been a small and little varied group, which has hitherto formed only one of the subsidiary elements in the flora.

These three possibilities may be represented graphically by life-lines as in Text-fig. 1.

Since the plant record of the Tertiary rocks does not furnish any real evidence in favour of one alternative rather than another, we must depend entirely on morphological evidence, and more especially on that afforded by the fructifications. The most plausible interpretation of the fructifications of these three genera, as we hope to show, is that they are reduced and highly modified forms of what we have called [1] 'pro-anthostrobili' (see p. 497). If this be the case, such evidence as there is strongly supports the first alternative; it is entirely incompatible with the second, and can only be regarded as in harmony with the third in a very slight degree. We assume, of course, one fundamental hypothesis, namely, that the Gnetales are monophyletic, a conclusion which, so far as we are aware, is not seriously challenged. Further, we believe it is only by first arriving at some clear idea of the status, so to speak, of the living genera, that we can begin the attack on the main problem of their relationships to the Angiosperms. The evidence that these two groups are distinctly related appears to us to be overwhelming and most convincing. Yet this by no means commits us to the view that one group gave rise directly to the other, but rather that both the Angiosperms and Gnetales sprang from a common ancestor. This ancestor, as yet unknown to us in the fossil state, we have already restored provisionally under the name of the Hemiangiosperm.[2]

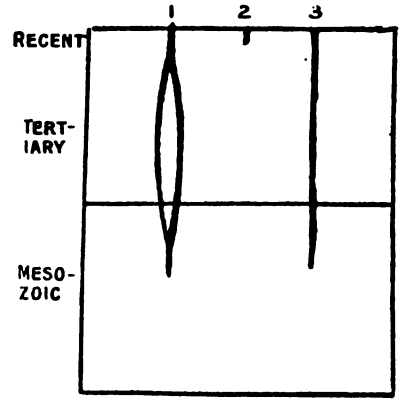


FIG. 1. Diagrammatic representation of the three alternative theories with regard to the past history of the Gnetales.

[1] Arber and Parkin ('07), p. 37.

[2] Ibid., p. 62, &c.

Historical.

In addition to reviewing the attempts that have been made to determine the place of the Gnetaceae in phylogenetic schemes, it may also be interesting and instructive to follow briefly the positions assigned to the three genera in the chief natural systems of classification from the time of Linnaeus onwards. We shall be chiefly concerned with *Ephedra* and *Gnetum*. *Welwitschia* (*Tumboa*) was not known until 1860. Sir Joseph Hooker,[1] three years later, by a thorough and masterly study of the material received from its discoverer, Dr. Welwitsch, unhesitatingly placed it in the Gnetaceae, as a new genus. Its position here has never since been questioned, and this conclusion is in reality, as we shall endeavour to show, the key to the status of the group.

Tournefort[2] founded the genus *Ephedra* in 1703. He placed it in his eighteenth class, set apart for trees and shrubs with apetalous flowers, The class following this also contained apetalous arborescent plants, which were, however, distinguished by the fact that their individual flowers were arranged in catkins. Thus *Ephedra* was not included in the amentiferous group, though placed in close proximity to it.

Linnaeus accepted Tournefort's genus, *Ephedra*, and established a new one, *Gnetum*,[3] for the Malayan tree, first described by Rumphius under the name *Gnemon domestica*.[4] In the fragment of a natural system of classification[5] left by Linnaeus, *Ephedra* is placed in the fifteenth group, the Coniferae, following the genus *Taxus*. The name *Gnetum* naturally does not appear, for it had not at that time been established. Passing to the younger De Jussieu, the first botanist to leave to us a complete natural system, we find in his scheme that *Gnetum*[6] is put in the Urticae, the third order of his last class, the Apetalae. The order following contains the catkin genera, then comes the fifth order, the Coniferae, in which *Ephedra* finds a place. It is grouped with *Casuarina* and *Taxus* in the first section of the order, which is characterized by a staminiferous calyx. The other section, distinguished by possessing staminiferous scales but no calyx, contains the plants now invariably included in the Coniferae. The two Gnetacean genera are thus set apart in different orders, the catkin family intervening. *Ephedra*, though placed in the Coniferae, is marked off, along with *Taxus* and *Casuarina*, as a distinct section. A point which deserves notice is De Jussieu's recognition of the presence of a calyx.

De Candolle, the next great systematist, in his 'Théorie Élémentaire de la Botanique', published in 1813, does not mention either *Ephedra* or *Gnetum*. Presumably in accordance with the opinions then current he

[1] Hooker ('63).
[2] Tournefort (1703), p. 53.
[3] Linnaeus (1767), p. 125.
[4] Rumphius (1750), vol. i, p. 181, Pl. 71.
[5] Linnaeus (1751), p. 28.
[6] De Jussieu (1789), pp. 406 and 411.

would also have kept them apart, like De Jussieu; for Bartling[1] in 1830 still adheres to this separation. He places *Gnetum* in his order, Chlorantheae, which directly follows the Piperaceae, while *Ephedra* appears in the Taxineae, one of the four groups into which he divides the Coniferae.

About this time Robert Brown published a paper 'On the Structure of the Female Flower in Cycadeae and Coniferae',[2] which marked an epoch in the progress of the study of floral morphology and taxonomy. The rise of the Gymnospermae as a separate group dates from this period, though the full significance of Brown's discovery of the naked megasporangia of the Cycads and Conifers, in contrast to the enclosed ovules of Angiosperms, was only gradually grasped later. In fact the complete separation of Gymnosperms from Angiosperms, as a whole, is comparatively modern. Brown[3] extended his standpoint of the lack of an investing carpel to *Ephedra* and *Gnetum*. He shows reason for regarding the style-like projection in the female flower of *Ephedra* as being ovular, rather than carpellary, in origin.[4] In our opinion he interpreted the morphological value of these envelopes more correctly than many of his successors, and his attitude towards the whole problem was thus considerably in advance of his time.

Robert Brown's views regarding the peculiarities of the gymnospermous ovule soon began to influence the position of *Ephedra* and *Gnetum* in classification. The merit of bringing these two genera together belongs to Blume. In a paper[5] published by him in 1834, he established a new order, the Gneteae, for *Ephedra* and *Gnetum*, characterized by possessing neither style nor stigma. He considered the group to be connected through *Ephedra* on the one hand with the Conifers, and on the other hand to *Casuarina*. Lindley apparently seized upon this conception of Blume's, changing the name of the order to Gnetaceae. In 1835[6] he placed *Gnetum* alone in it, but the year following[7] he included *Ephedra* also.

The system of Endlicher[8] now claims a passing notice. His Gymnospermae, ranking as a group equivalent to the Apetalae or Gamopetalae, consist of one class, the Coniferae, which is subdivided into four orders—Cupressineae, Abietineae, Taxineae, and Gnetaceae. Here we observe that the Gnetaceae are regarded as a comparatively subordinate group of Gymnosperms, nearly related to the Taxineae.

Brongniart,[9] though the first systematist to raise the Gymnosperms to a rank equivalent to that of the Dicotyledons, still kept the Gnetaceae in the same relatively subordinate position as Endlicher.

[1] Bartling ('30), pp. 86 and 96.
[2] Brown ('27).
[3] Ibid., p. 453.
[4] Ibid., p. 457.
[5] Blume ('34), p. 101.
[6] Lindley ('35), Pl. 1686.
[7] Lindley ('36), p. 311.
[8] Endlicher ('36), p. xi.
[9] Brongniart ('43), p. xxxii.

Parlatore,[1] author of the Gymnosperm section of De Candolle's 'Prodromus', divided the Gymnospermae into three groups of equivalent rank, viz. the Gnetaceae, Coniferae, and the Cycadaceae; a conclusion which was also adopted by Bentham and Hooker.[2] Here for the first time we find the Gnetaceae clearly separated from the Conifers. From this period onwards the two genera, with the addition of *Welwitschia* in 1863, have been constantly associated together as a separate group of naked seed-plants. The more modern trend of opinion, as we shall see, has been to isolate them more and more, and to raise the taxonomic value of the group to that of a class, equivalent in rank to the Coniferales and Cycadales.

Reviewing the main steps by which the Gnetacean genera have risen in schemes of classification, it is to be noticed that, on the one hand, their inclusion in the Dicotyledons became prohibited when the significance of the naked ovule was realized, and on the other hand the presence both of floral envelopes simulating perianths, and of male sporophylls, showing a likeness to Angiospermous stamens, as well as the dicotyledonous type of foliage possessed by *Gnetum*, precluded their retention in the Coniferae. They have thus, in the history of classification, hovered as it were midway between the Coniferae and the Dicotyledons.

Passing from taxonomy to phylogeny, a paragraph in Lindley's 'Vegetable Kingdom',[3] though written before the publication of 'The Origin of Species', may be worth quoting. 'There exist, however, a few plants, not very similar to each other in appearance, bearing the names *Gnetum* and *Ephedra*, in which we find precisely the structure and habit that would be wished for by a theorist searching for evidence to bring Gymnogens into communication with true Exogens; for one of them has all the appearance of a Chloranth and the other of a Casuarina; and yet both retain the true peculiarities of Gymnogens.'

Parlatore[4] suggested that a connexion existed between the Gnetaceae and the Amentiferous and Piperaceous families. Agardh[5] concluded that a close relationship was to be found with the Loranthaceae, a view which was received favourably by Henfrey.[6] Hooker[7] also, from his study of *Welwitschia*, showed that this plant, in common with *Gnetum* and *Ephedra*, presents some very curious points of resemblance to both the Loranthaceae and Santalaceae. Strasburger,[8] referring to the functionless ovule of the male fructification of *Welwitschia*, remarked that such a flower calls to mind the hermaphrodite flower of the higher Phanerogams, and that doubtless here are to be derived certain Angiospermous groups, such as the Loranthaceae.

[1] Parlatore ('68), p. 347.
[2] Bentham and Hooker ('83), vol. iii, p. vii.
[3] Lindley ('53), p. 232.
[4] Parlatore ('67), p. 100, ('68), p. 346.
[5] Agardh ('58), pp. 317–20.
[6] Henfrey ('59), p. 299.
[7] Hooker ('63).
[8] Strasburger ('72), p. 243; see also the plate opposite p. 264.

More modern researches, however, have tended to widen greatly the gap between the Gnetaceae and the Angiosperms. Bower,[1] from his examinations of the germination and seedling structure of *Welwitschia* and *Gnetum Gnemon*, concluded that the Gnetaceae are a more natural group than has hitherto been supposed, and that *Gnetum* is, in reality, scarcely more closely comparable with the Angiosperms than the other two genera. Lotsy,[2] from his researches on *Gnetum*, came to the conclusion that in all probability no Angiosperm had a Gnetacean ancestor, but that the Gnetaceae arose quite independently. He would derive them directly from heterosporous Pteridophytes, as a separate line quite distinct from those giving rise to the Gymnosperms and Angiosperms. Lignier,[3] in a theoretical paper on the relationship of the fructifications of the Gymnosperms to the Angiosperms, finds that the Gnetaceae cannot have been intermediate between these two large groups.

Land,[4] in his recent paper on *Ephedra trifurca*, remarks that 'there is no proof that the Gnetales have been derived from or are directly related to any living group'. If they are 'related to the Coniferales at all, it must be to the Taxaceae'. He thinks the Gnetales are better regarded as a modern group.

At the present time, however, some botanists are not content to abandon all hope of connecting the Gnetaceae and the Angiosperms. The points of resemblance are too many and too striking for homoplasy to afford a sufficient explanation. Some quite recent attempts[5] to link these two groups may now be briefly considered.

Since the publication of our previous paper, Professor Wettstein[6] has elaborated for the first time the theory on which depends the belief that the hermaphrodite flowers of the Angiosperms, with a conspicuous perianth, are derived from unisexual, apetalous forms. He traces the origin of the Polypetalae from the Gymnosperms, making use of *Ephedra* and *Casuarina*, as connecting links. Porsch[7] also finds support for this view by an appeal to embryological evidence.

Hallier[8] at one time attempted to revive the supposed relationship between the Gnetales and the Loranthaceae, a view which we gather he has recently abandoned.

We may thus conclude from this historical sketch that the balance of opinion at the present time appears to be against the views, either that the Angiosperms have sprung from the Gnetales, or that the Gnetales hold an intermediate position between Gymnosperms and Angiosperms.

[1] Bower ('82), p. 298.
[2] Lotsy ('99), p. 100.
[3] Lignier ('03).
[4] Land ('07), p. 288.
[5] A short paper by Miss Benson ('04) on this subject was referred to on p. 33 of our previous paper ('07). The author informs us that we have misunderstood her argument, which we much regret.
[6] Wettstein ('07[1]), vol. ii, part ii, first half, p. 203, and ('07[2]).
[7] Porsch ('07), pp. 14–15.
[8] Hallier ('07), p. 497.

Yet it is undoubted that, of all Gymnosperms, the Gnetales stand nearest to the Angiosperms. The number of characters in which they approach the Angiosperms is such as to render improbable, for example, Lotsy's conclusion that they have no nearer common ancestor than the Pteridophytes.

Several attempts have also been made, as we have seen, to link the Gnetales directly with certain existing Dicotyledonous families. None of these have as yet carried conviction, nor do we think it likely that these views will gain ground in the near future.

The deductions drawn from the superficial resemblance of the aggregates of the fructifications, and certain vegetative features, to the amentiferous and some other apetalous Dicotyledons have in our opinion been pushed too far. The naked ovule, alone, is a bar to any direct linkage. It remains naked, despite all attempts to clothe it in a carpellary covering.

The old suggestion of a close relationship between the Gnetales and the Coniferae, especially to *Taxus*, scarcely demands attention now.

The Gnetales thus at the present time remain largely a phylogenetic puzzle. They are Gymnosperms, but, as we have seen, they undoubtedly possess strong Angiospermous affinities. Yet the attempts which have hitherto been made to derive the Angiosperms from them have not, it must be admitted, the merit of simplicity.

Morphological Considerations.

In the present consideration we shall confine our attention chiefly to the fructifications, for, just as in the case of the Angiosperms, we regard these as forming the crux of the whole problem. Conclusions in regard to anatomy, or even those derived from a study of the embryo-sac, however interesting, we look upon in this case as of less phylogenetic value.

We propose to inquire whether the Strobilus Theory, as outlined in our earlier paper in reference to the Angiosperms, will not afford a satisfactory explanation of the Gnetales. We may, however, first recapitulate the new terminology introduced in that memoir, which we propose to apply here also.

We restrict the use of the term 'flower' to the Angiosperms. The fructifications of the Gnetales, often referred to as 'flowers', we shall speak of as strobili.[1] The inflorescences, which in the case of *Welwitschia* and *Ephedra* have been frequently termed cones, we shall refer to simply as aggregates of strobili. We prefer also the more non-committal terms, micro- and megasporophylls, in place of stamens and carpels, and amphisporangiate and monosporangiate for hermaphrodite and unisexual.

[1] We have already shown that the typical, and more primitive Angiospermous flowers are strobili [Arber and Parkin ('07), p. 36].

If we institute a comparison between the fructifications of the three genera of the Gnetales, we arrive at the following conclusions, each of which merits separate consideration.

1. One fructification is amphisporangiate, the others are all monosporangiate.

2. Each fructification, in addition to microsporangia or a megasporangium, contains other organs, which we, with others, regard as constituting a perianth.

3. The fructifications are grouped in dense, sometimes complicated, aggregates.

4. The microsporophylls and microsporangia bear some likeness to Angiospermous stamens, but at the same time the differences are no less marked.

5. There is no trace of a megasporophyll, comparable to a carpel. The female organ is a solitary terminal megasporangium, with one or two integuments.

The Amphisporangiate Condition Primitive.

The amphisporangiate condition is undoubtedly present in the male fructification of *Welwitschia*, though the megasporangium is no longer sexually functional. Hooker, himself, was much impressed by his discovery of the functionless ovule in this fructification, which he considered gave great weight to the view that the Gnetaceae had arisen from 'hermaphrodite-flowered plants'.[1]

Coulter and Chamberlain remark that 'while the flowers are functionally monosporangiate, there is evidence of their derivation from a bisporangiate condition'. The same authors add that 'the whole structure is puzzling and anomalous among Gymnosperms, for it seems to indicate derivation from a perfect flower'.[2]

The male fructification of *Welwitschia*, in our opinion, is the key to the floral' morphology of the Gnetales. We regard it as a strobilus, possessing all the peculiarities of that particular type, which in our earlier paper we termed an *Anthostrobilus*. The anthostrobilus[3] was defined as an axis bearing perianth members, microsporophylls and megasporophylls, arranged in a characteristic order, the female organs being invariably placed above the microsporophylls. One variety of the Anthostrobilus, in which the ovules alone perform the task of pollen-collection, was distinguished as a *Pro-anthostrobilus*. The strobilus of the Bennettiteae is an example of a pro-anthostrobilus. On the other hand, the *Eu-anthostrobilus* or Flower (the latter term being used only in a rigidly restricted sense) is typical

[1] Hooker ('63), p. 24.

[2] Coulter and Chamberlain ('01), p. 120.

[3] Arber and Parkin ('07), p. 37.

of the Angiosperms. Here the megasporophylls carry on the work of pollen-collection.

On our view the male fructification of *Welwitschia* is a complete pro-anthostrobilus. It possesses organs which, in common with other botanists, we regard as constituting a perianth. Above these, microsporophylls occur, and although no megasporophylls are to be found at the apex of the strobilus, a megasporangium is present. The strobilus is obviously a pro-anthostrobilus, for the office of pollen-collection would be entirely performed by the ovule, were it functional.

The male strobilus of *Welwitschia* is in our opinion the most primitive of the Gnetalean fructifications. The female, and both the male and female strobili of *Ephedra* and *Gnetum*, have been derived from it by reduction; one set of organs, either male or female, being entirely suppressed. These fructifications are therefore very reduced pro-anthostrobili, which have originated from amphisporangiate forms. Thus the application of the Strobilus Theory to the Gnetales offers a simple explanation of the peculiarities of their fructifications, which have hitherto proved so puzzling. We now proceed to inquire in some detail how far the evidence will support this view.

The Perianth.

In the fructifications of all three genera the sexual organs are subtended by and enclosed in envelopes (Text-fig. 2), the morphological interpretation of which has given rise to much divergence of opinion.

The view that these structures in the male fructifications, and the outer series in the female, are of the nature of a perianth is a very old one. Linnaeus[1] referred to the envelope of *Ephedra* as a fleshy calyx. De Jussieu separated *Ephedra*, with *Casuarina* and *Taxus*, from the rest of the Coniferae on the presence of a 'staminiferous' calyx.[2]

In regard to the male fructifications, these envelopes might either be looked upon as of the nature of a perianth, or merely as bracts with no phylogenetic significance. In the case of the female, all of them might be held to be equivalent to integuments, or the outer series, the homologues of either bracts, perianth, or even megasporophylls. It is from these possibilities that we have to choose, and it may be worth while briefly reviewing previous opinions in this connexion.

Robert Brown,[3] a great authority, decided as follows: 'In *Ephedra*, indeed, where the nucleus is provided with two envelopes, the outer may, perhaps, be supposed rather analogous to the calyx or involucrum of the

[1] Linnaeus (1751), p. 75. [2] De Jussieu (1789), p. 411.
[3] Brown ('27), p. 455.

male flower, than as belonging to the ovulum; but in *Gnetum*, where three envelopes exist, two of these may, with great probability, be regarded as coats of the nucleus.'

In regard to *Welwitschia*, Hooker[1] considered that a perianth is present in both the male and female fructifications. He also showed that the outer envelope in the former is absent in *Ephedra* ♂, while the inner is identical in appearance and position in both genera. He further states[2] that, in the female fructification, the perianth 'corresponds to the outer

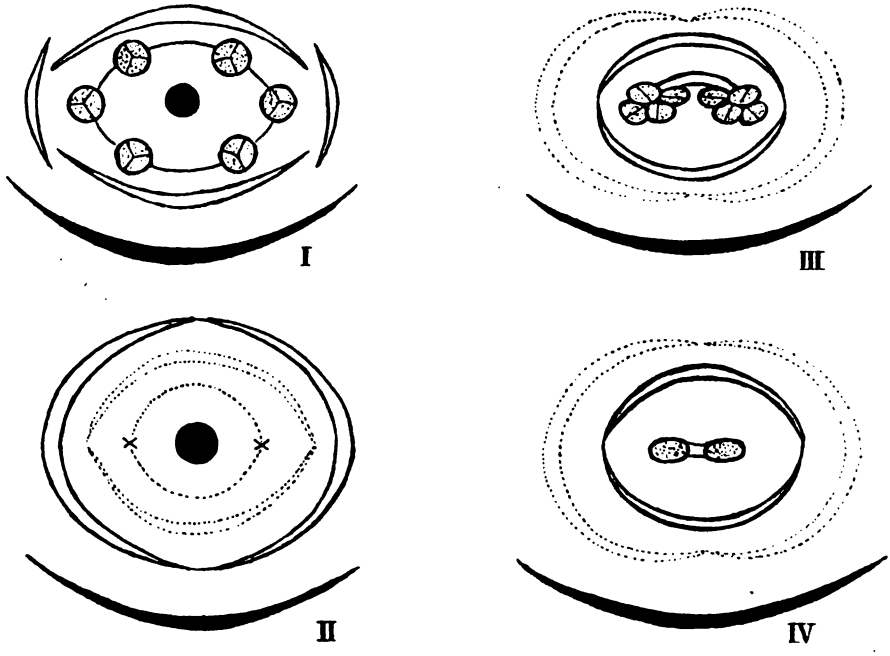


FIG. 2. Diagrammatic relative plans of the Gnetalean strobili, as here interpreted. Each strobilus is subtended by a bract. I. *Welwitschia* ♂, possessing a biseriate perianth, microsporangia, and a megasporangium. The strobilus is essentially dimerous. II. The ♀ strobili of *Welwitschia*, *Gnetum*, and *Ephedra*. The inner perianth whorl and the microsporangia are suppressed. III. *Ephedra distachya*, Linn. ♂. The outer perianth whorl and the megasporangium are suppressed. IV. *Gnetum* ♂, similar to III, but differing as regards the microsporangia.

(lateral) leaflets of that of the hermaphrodite' flower, and also 'to the outer coriaceous covering of the ovule in *Ephedra* and in *Gnetum*' (Text-fig. 2). This conclusion offers a simple explanation of the whole difficulty, and it is one with which we find ourselves in agreement, believing as we do that *Welwitschia* affords the real clue to the correct interpretation of the homologies of the Gnetalean fructifications.

As is well known, Strasburger,[3] in his attempts to interpret the female Gnetalean fructifications in terms of the Coniferous cone, has naturally seen

[1] Hooker ('68). [2] Ibid., p. 27. [3] Strasburger ('79), p. 120.

no homologue of a perianth. In 'Die Angiospermen und die Gymnospermen' he concluded that all the envelopes are integuments, in which case *Gnetum* may possess three. But in our opinion the strong argument against this view is that in the male fructifications of both *Gnetum* and *Ephedra* (Text-fig. 2) an envelope is present, which cannot be integumentary, but which is obviously of the same morphological value as the outer envelope of the female fructification found in all three genera. For from the male strobilus of *Welwitschia*, where this perianth is less reduced, and consists of two cycles of two members each, we can easily derive the single perianth whorls found in all the female strobili, and also in the male of *Ephedra* and *Gnetum*. On Strasburger's view the male fructification of *Welwitschia* is unintelligible, and has no phylogenetic significance.

The Aggregates of Strobili.

The fructifications of the Gnetales do not, as is well known, occur as isolated reproductive shoots, but are aggregated into dense groups, which in *Welwitschia* and *Ephedra* are somewhat cone-like in external appearance. These aggregrates of strobili may be further collected together, apart from the purely vegetative organs, to form a system of reproductive shoots, which may be closely compared with complicated inflorescences found among Angiosperms. We regard such aggregates as indicating a high degree of evolution, and a far from primitive state. For we would apply here the same reasoning as in the case of the Angiosperms, where we have shown that the presence of a dense and complicated inflorescence, correlated with a much reduced perianth and unisexual flowers, may be recognized as a sign of a highly evolved state, derived originally from plants possessing solitary, hermaphrodite flowers with a conspicuous perianth.[1] The Gnetales thus appear to us to afford another instance in which the tendency of specialization has been in the direction of the inflorescence rather than of the individual flowers, and here, as is so often the case among Angiosperms, it is correlated with a great reduction in the less essential floral organs, and with the evolution of unisexuality.

The aggregates of *Ephedra* are fairly simple, the male strobili being arranged in compact spike-like groups, each containing several strobili. These spike-like groups may be clustered together on neighbouring branches. The female fructifications are also borne in similar groups, which however contain only 1–3 strobili. In some Ephedras female strobili may occur at the apex of the male spike-like branches.[2]

The aggregates in *Welwitschia* are essentially similar to those of *Ephedra*, though rather different in size and appearance, the female spike-groups being very much larger, and recalling somewhat the cones of the Coniferae. In both the male and female aggregates, several strobili occur.

[1] Arber and Parkin ('07), pp. 38–42.

[2] Wettstein ('07[2]).

In *Gnetum*, the condition of affairs is very different. The strobili are borne on special branches, which at first sight have some resemblance to slender, solitary spikes or to a panicle of spikes. Each fertile branch, however, bears numerous strobili arranged in whorls, subtended by pairs of bracts. On the female branches, 3–8 strobili are found in the whorl, while on the male, as many as 40 may occur. Numerous hair-like structures occur between the strobili. It would appear probable that the real 'inflorescence', or rather the aggregation of strobili in *Gnetum*, is of a compound nature and a complicated type, and at the same time much compressed. The whole may be not inaptly compared with the compressed cymes of certain Labiates, such as *Lamium*.

It is not our object to consider these aggregates here in detail. It is significant, however, to note that the reduced fructifications are arranged in dense, and sometimes complicated, aggregates; a combination of circumstances also met with among some of the more highly modified Angiosperms, such as the Amentiferae. That such a comparison implies nothing more than a parallelism of development is apparent when we bear in mind that we are here contrasting pro-anthostrobili with eu-anthostrobili.

We may also point out that as regards the strobili themselves, apart from their aggregates, the parallelism between the two groups is very close. Those of *Ephedra* and *Welwitschia* find their analogues in the Salicaceae. The female fructifications of *Welwitschia* correspond to those of *Populus* in that the strobili are aggregated into spikes, each strobilus being subtended by a bract. A gamophyllous perianth is also present (Text-fig. 2). They differ, however, in the absence of megasporophylls and in the fact that solitary ovules alone occur. Both the male and female strobili of *Ephedra* also correspond with the flowers of *Populus*, with the same differences, and with the additional exception that the microsporophylls are less numerous.

While not wishing to press too closely a comparison between the two groups, which we think is founded merely on a parallelism of development, we may, however, point out the interesting fact that an almost exact homoeomorph of the male strobilus of *Welwitschia* has recently been discovered in the amphisporangiate flower of an Indian Poplar, *Populus glauca*, Haines.[1] The flower of this species corresponds very closely with the male pro-anthostrobilus of *Welwitschia*, except that the number of stamens is greater, and that the female organ is functional.

The Microsporophylls and Microsporangia.

We may now turn our attention briefly to the male organs of the Gnetales, which, though differing unmistakably from the stereotyped form of the Angiospermous stamen, yet more closely resemble it than the

[1] Haines ('06); see also Arber and Parkin ('07), p. 41.

microsporophylls of other Gymnosperms, either recent or fossil. There has been considerable divergence of opinion as to what corresponds exactly to a microsporophyll in these plants, as distinct from the microsporangia, and also as to the number of sporophylls present in each genus. The morphology appears to us to be rendered difficult by the great reduction and cohesion which has taken place. In *Ephedra* and *Gnetum* reduction has been carried to such a degree that a monosporangiate fructification has resulted, which further obscures the homologies.

The male flower of *Welwitschia* seems to present the least difficulty. The male organs here at least are lateral structures. Hooker[1] considered that six, partly united stamens, in one whorl, are present, each possessing a trilocular anther. This hexandry, he says, is a departure from the binary arrangement found elsewhere in this plant. McNab[2] disagreed with Hooker, and showed that a dimerous symmetry prevails, for, in development, the six male organs arise from two primordia placed laterally, alternating with the inner perianth whorl. Strasburger,[3] though originally holding a different view, adopted McNab's interpretation in 1879. We are also in agreement with this conclusion.

The first point to be considered is the homology of these organs in the three genera. Here again we find that *Welwitschia* affords the clue. In that genus there arises from each of the two primordia a structure resembling a microsporophyll, and bearing three, stalked synangia. We do not propose to express any opinion as to whether this organ is derived by the fusion of three separate microsporophylls, or whether it is more truly to be regarded as a single, branched sporophyll. For the sake of clearness in comparison, we propose to speak of it as a *unit*.

The male organs of all three genera can, on our view, be interpreted as derived from two such units. In *Welwitschia*, we find two units, each bearing three, stalked synangia. In *Ephedra* and *Gnetum* the two units are united, though in some species of the former genus they remain free for some distance above.

This theory is that which has been clearly worked out by Thibout,[4] who has given an admirable explanation of the homologies of the male organs in all three genera, and has shown the close connexion existing between them. Starting from *Ephedra distachya*, Linn. (Text-fig. 3, I), where the two units have each four synangia, which either cohere entirely, or remain somewhat free at the apex, we pass next to *Ephedra fragilis*, Desf. (Text-fig. 3, II), where one synangium (the inner lateral marked *d* in Text-fig. 3, I) of each unit is suppressed. This is the condition found in *Welwitschia*, where, however, the synangia are trilocular and stalked (Text-fig. 3, V). It may be pointed out, however, that in *E. fragilis*, this

[1] Hooker ('68), pp. 22 and 41.
[2] McNab (78), p. 508.
[3] Strasburger ('79), pp. 133-4, footnote.
[4] Thibout ('96), part iii, Pls. XV and XVI.

condition of affairs is not constant, for in some cases two of the inner synangia may fuse together, or other variations may be found.

The third stage is seen in *E. altissima*, Desf. (Text-fig. 3, III), where three of the synangia of each unit are suppressed (those marked *a*, *b*, *d* in Text-fig. 3, I) and a single synangium (*c*) remains. This is also the condition of affairs met with in *Gnetum*, where, however, the two synangia have each been further reduced to a single loculus (Text-fig. 3, IV).

On the above view the axial position of the male organs, in *Ephedra* and *Gnetum*, on which great stress has been laid in some quarters, presents

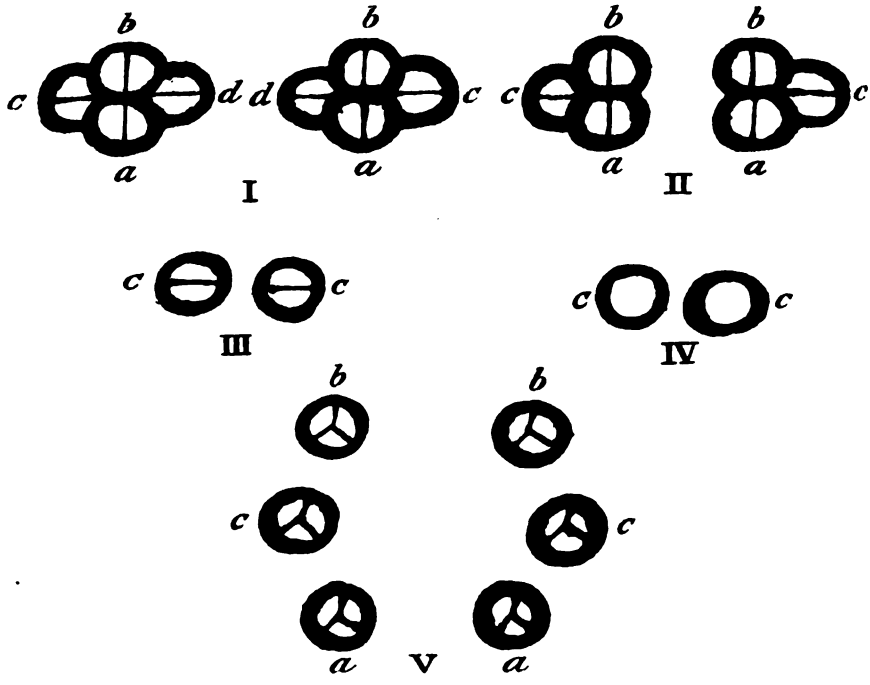


FIG. 3. Diagrammatic representation of the microsporangia of the Gnetales. I. *Ephedra distachya*, L.; the two 'units', each composed of four bilocular synangia, lettered *a–d*. II. *E. fragilis*, Desf., the two 'units' of three bilocular synangia, the synangium *d* being suppressed. III. *E. altissima*, Desf., the two 'units' each consisting of a bilocular synangium. The synangia *a*, *b*, and *d* are suppressed. IV. *Gnetum*; two units, each consisting of a unilocular synangium. V. *Welwitschia*; two 'units' similar to II, but the synangia are trilocular.

no difficulty. Through the suppression of the female, they have come to occupy a central position, and, being more or less united, they naturally form a median column.

The male organs of the Gnetaceae do not appear to us to present any transition to the Angiospermous stamen. They seem to have equally departed from the original type, though along different lines. The presence of synangia, in the case of *Ephedra* and *Welwitschia*, is a point in common,

however, on the view that the stamen of the Angiosperms is a 'sporangiophore bearing two synangia'.[1]

As regards the evolution of these organs we are inclined to derive them by reduction and fusion from more complicated sporophylls bearing many synangia. Such sporophylls are known in *Bennettites*, and were also characteristic, as we have shown, of the hypothetical Hemiangiosperms, the direct ancestors of the Angiosperms, though in a more reduced form.[2]

Wieland[3] has pointed out that there is no 'unbridgeable gap between the staminate disk of *Cycadeoidea* and that of *Welwitschia*'. This explanation will also be found to account for the variation in the number of the synangia, and of their component sporangia, on the basis of a progressive reduction, which in the case of *Gnetum* has reached its furthest limits.

The Megasporangium.

The morphological interpretation of the female fructification is as puzzling as that of the male. There is only a single megasporangium (ovule) in all three genera, which is situated terminally, and is surrounded by two, or in *Gnetum* by three, envelopes. We have already concluded that the outer envelope is in reality a perianth. In *Ephedra* and *Welwitschia*, the inner is evidently integumentary, as it has been generally regarded. In *Gnetum*, however, there is a further difficulty as to the homology of the middle envelope. The innermost corresponds so exactly to the inner covering of *Ephedra* and *Welwitschia* in its adaptation as a spout-like micropyle, that there is every reason to consider it as homologous.

We thus adopt the usually accepted conclusion, that none of the envelopes are equivalent to a megasporophyll (carpel). That some sort of foliar structure bearing the megasporangium was present in the more primitive Gnetaceae appears to us to be highly probable. The apparently terminal, 'cauline' ovule possessed by the three existing genera is not, however, a point which causes us hesitation. We agree with Goebel,[4] as opposed to the older school of morphologists, in regarding leaf-borne and axis-borne organs as not necessarily of a different morphological value, when in their other peculiarities they appear to be similar. It therefore follows that it is quite legitimate to trace phylogenetically such differently situated organs to the same place of origin, and to follow 'the path by which this axial position has been acquired'.

It must be remembered that the megasporophylls of the Gnetaceae were never so fundamentally essential as the Angiospermous carpels, for in the former group the pollen-collection was in all probability always per-

[1] Arber and Parkin ('07), p. 68.
[2] Ibid., p. 63, Text-fig. 4.
[3] Wieland ('06), p. 245.
[4] Goebel ('05), part ii, p. 556.

formed by the megasporangium itself; an inference supported by the presence, in the three genera, of a long, tubular micropyle, the adaptation of which is obvious. Consequently in plants pollinated in this manner where reduction has been carried to extreme limits, it is not surprising to find that the megasporophyll has totally disappeared, and that the ovule is now borne on the axis. On the other hand, in the most reduced Angiospermous flowers, in which cauline ovules occur, the closed megasporophyll must persist to continue the task of pollen-collection.

Female Gametophyte (Embryo-sac).

In recent years attention has been largely focussed on the embryo-sac. The papers of Lotsy,[1] Land,[2] Pearson,[3] and Berridge and Sanday,[4] have shown that, on the one hand there is a great reduction in the gametophyte, when compared with other Gymnosperms, and on the other a notable dissimilarity between the embryo-sacs of all three genera. Such facts have been interpreted as evidence that the three genera are not so closely allied to one another as has been supposed, and they have further been regarded as likely to throw light on the puzzling homologies of the stereotyped form of the Angiospermous embryo-sac. In *Ephedra* the gametophyte is decidedly the least reduced. Typical Gymnospermous archegonia are present. There is, however, some morphological distinction between the upper (reproductive) and the lower (vegetative) region of the mature embryo-sac.

In *Gnetum Gnemon* the sac is also markedly differentiated into two parts, but no archegonia have as yet been found at the upper (micropylar) end. Here free nuclei only occur, which apparently are all potentially eggs. The lower (antipodal) portion consists of a more compact tissue, which continues its growth after fertilization. In other species of *Gnetum*, Karsten[5] has observed further stages in the reduction of the gametophyte, and has shown that the 'endosperm' is not developed until after fertilization—a striking parallelism to Angiosperms, and a contrast to other Gymnosperms.

The embryology of *Welwitschia* has been considered to be more or less intermediate between *Ephedra* and *Gnetum Gnemon*, but Pearson's researches[6] do not favour this view. The embryo-sac in *Welwitschia* appears to be highly modified on somewhat different lines.

In respect to the development of the embryo, Coulter and Chamberlain[7] regard *Ephedra* as standing nearest to the other Gymnosperms. Bower,[8] however, from his study of *Gnetum Gnemon*, concludes that 'In

[1] Lotsy ('99). [2] Land ('04) and ('07). [3] Pearson ('06).
[4] Berridge and Sanday ('07). [5] Karsten ('93). [6] Pearson ('06), p. 297.
[7] Coulter and Chamberlain ('01), p. 132. [8] Bower ('82), p. 298.

the development of its embryo it obviously follows the type of the Coniferae, and indeed approaches them in some respects more nearly than either *Ephedra* or *Welwitschia*'.

We are inclined to think that too much weight has been attached to these embryological dissimilarities from the point of view of the relationship of the three genera to one another. The great contrast between the embryo-sacs of *Gnetum* and *Welwitschia* on the one hand, and *Ephedra* on the other, is no doubt remarkable, and, apart from other morphological considerations, it might be perfectly legitimate to conclude that their relationships were distant. But it is unwise to reason phylogenetically from the evidence of one organ, to the neglect of others, even though that organ be the female gametophyte. We think the sum total of the agreements between the three genera is too strong to permit of the complete separation of *Ephedra* from *Gnetum* and *Welwitschia*.

We would suggest that the immediate ancestors of the Gnetales had a fairly typical Gymnospermous embryo-sac, which has more or less persisted in the case of *Ephedra*, but which in *Gnetum* and *Welwitschia* has undergone considerable reduction with peculiar modifications.

We now turn to the question of Angiospermous affinities. The suppression of the archegonia, the formation of a storage tissue after fertilization, and the discovery of nuclear fusions quite recently made by Berridge and Sanday, are all facts which have been regarded as rendering hopeful the possibility of interpreting the Angiospermous embryo-sac by means of the Gnetales. Though not wishing to seem to depreciate in the least the great value of these embryological observations, we are doubtful if this hope will be realized.

Miss Sargant,[1] in her paper in the April number of the Annals, has discussed the Gnetalean embryo-sac, and has pointed out that no tissue 'in *Gnetum*, nor, so far as we know, in *Welwitschia*, can be considered as the direct representative of the Angiospermous endosperm'. In other respects also the resemblances appear to us to be more superficial than real. While no doubt in the history of the evolution of the embryo-sac of both *Gnetum* and *Welwitschia*, and also in the Angiosperms, a corresponding stage was at one time reached when the archegonia became suppressed, yet since then further modifications have taken place along different lines. In the case of the Angiosperms the chief modern speciality has been the formation of endosperm as a result of double fertilization, while in the Gnetalean genera special features, such as the prothallial tubes, have been evolved.

The outstanding characteristics of the embryo-sacs of *Welwitschia* and *Gnetum* as compared with *Ephedra* may in some measure be analogous to those of *Peperomia*[2] as compared with *Piper*, and are thus more of

[1] Sargant ('08), p. 135.

[2] Johnson ('05) and ('07).

the nature of anomalies than typical of the Gnetales of the past. The embryology of the Gnetales, as a whole, is still imperfectly known, and it would not surprise us if further research should result in bringing the three genera into closer agreement as regards their embryo-sacs.

Fossil Evidence.

In the previous section we have shown that the most reasonable interpretation of the fructifications of the Gnetales appears to us to be that they are very reduced pro-anthostrobili, aggregated into dense clusters, which have often a spike-like habit. If this be true, it follows that at one time more primitive members existed, bearing typical pro-anthostrobili, which possessed a perianth and both male and female organs, the latter arranged above the male. On this view the Gnetales are the last survivals of a race, the strobili of which must have presented much variety in form and detail, and consequently the group itself must have been of some size and complexity. We ought, therefore, to find in the rocks evidence of such a stock. Yet such is not the case. So far as we are aware, no reliable evidence exists at present of fossil Gnetales. Solms has already expressed a similar conclusion in his Fossil Botany.[1]

It is true that some authors in describing fossil plants, especially of Tertiary age, have attributed a few remains to this group. In most cases these determinations have been founded on very obscure, badly preserved, or fragmentary impressions, which are not worthy of consideration in this connexion. The fossils termed *Ephedrites Sotzkianus* by Unger,[2] and *E. antiquus* by Heer,[3] are such examples. The *Ephedra Johniana*[4] and *E. Mengeana*[5] of Goeppert, fossils preserved in the amber of the Baltic, are now referred by Conwentz[6] to the genus *Patzea* of the Loranthaceae, and are certainly not members of the Gnetales. The Palaeozoic seeds referred to the genera *Gnetopsis*, *Samaropsis*, &c., which Renault[7] regarded as belonging to the Gnetales, are not now believed to have any connexion with that group. The former are with little doubt the seeds and cupules of a Pteridosperm.

Although the Gnetales are unknown to us in the fossil state, we are by no means precluded *ipso facto* from the conclusion that the group may have once flourished much more vigorously than at the present day. The progress of Palaeobotanical research during the past forty years has taught us that many groups, such as the Equisetales and the Ginkgoales, of

[1] Solms ('91), p. 126.
[2] Unger ('51), p. 159, Pl. XXVI, Figs. 1–11.
[3] Heer ('76), p. 82, Pl. XIV, Figs. 7, 24–32, and Pl. XV, Figs. 1 *a* and 1 *b*.
[4] Goeppert ('88), p. 47, Pl. XVI, Figs. 243–5, 247, 247 *a*.
[5] Ibid., p. 48, Pl. XVI, Figs. 248–50.
[6] Conwentz ('86), pp. 136–9, Pl. XIII, Figs. 8–20.
[7] Renault ('85), vol. iv, pp. 176–83.

which but few descendants survive, were once dominant constituents in the floras of the past. In many cases we have discovered relics of their former greatness in the Palaeozoic or Mesozoic rocks. It may therefore be asked how is it that if, as we suppose, the Gnetales were a fairly abundant element in a former flora, we can produce no evidence of them in the fossil state? The answer is to be found in the fact that our present knowledge of the real affinities of Cretaceous and Tertiary plants is extremely weak, and far less satisfactory than that of the Palaeozoic or even of the Mesozoic floras. Practically no petrifactions are known, with the exception of much silicified wood of Angiospermous or Gymnospermous affinities, which does not contribute to the question either one way or the other. Further, even impressions of fructifications have hitherto proved to be extremely rare, and very unsatisfactory from this point of view. The few flowers preserved in the Oligocene amber stand quite alone as affording reliable evidence in this direction. By far the larger number of specimens, which have been described from the Cretaceous and Tertiary rocks, consist solely of impressions of leaves, in the great majority of cases quite detached. The only criteria which they present, as indicative of their affinities, are the shape of the leaf and the details of the nervation, both, in our opinion, extremely unsatisfactory indices of systematic position, where they have to be relied upon alone.

Let us imagine for a moment that it was known, from the evidence of fructifications preserved in amber, that the Gnetales really did exist in the early Tertiary period. What would then be our attitude towards the Cretaceous and Tertiary leaf impressions? One of the three living genera is practically leafless. Another, *Welwitschia*, is so extremely specialized, that its leaves give us no certain clue as to what was the type of the foliage characteristic of the group as a whole. There remains *Gnetum*, with its opposite leaves, which in form and nervation recall exactly those of certain Dicotyledons. Thus, so far as the living members of the group are concerned, the only hint they give us, as to what was possibly a typical Gnetalean leaf, is one which coincides exactly with that of a Dicotyledon. Were the fact known from fructifications preserved in amber, that the Gnetales *did* exist throughout the Tertiary period, we should then be led inevitably to the conclusion that some of the reticulately-nerved leaves of the Tertiary rocks were, in all probability, not the leaves of Dicotyledons, but of the Gnetales.

That such is the case we believe to be highly probable, although we have no undoubted evidence of the group in the fossil state. The flowers preserved in amber, and those known as impressions, which are sufficiently well preserved to be more or less recognizable, are, after all, extremely few in comparison with the number which have perished completely, and the negative evidence in this case is even more untrustworthy

than usual. Not only are the fructifications of the Gnetales highly evolved structures, and far from primitive,—a conclusion which also points to the existence of the group in the past,—but the foliage of *Gnetum*, so Dicotyledonous in every respect, also strongly supports this view. Thus the leaves of past members of the Gnetales may have been already described, but confounded with those of Dicotyledons, and to this may possibly be due the paucity of fossil evidence of the former group.

The Ancestors of the Gnetales.

We may now consider the question how far the evidence will permit us to restore the past history of the Gnetales.

The occurrence at the present time of three genera alone, one of which is monotypic and restricted in the highest degree as regards its distribution, and the fact that these genera, when compared, present such diverse features, are not in favour of the view that the group has had little or no past history. The facts are much more intelligible on the supposition that we are dealing here with three survivals of a group, which was once of considerable complexity but has now almost perished entirely. But a stronger argument, pointing in the same direction, is to be found in the interpretation of the Gnetalean fructifications advanced here. It has been shown that the application of the Strobilus Theory to these fructifications offers a simple explanation of their peculiarities. We have seen that on our view these may be regarded as reduced anthostrobili, derived originally from typical amphisporangiate pro-anthostrobili, with a well-marked perianth. A strong point in favour of this conclusion is that these reduced anthostrobili are aggregated into dense groups or 'inflorescences', which is exactly what we met with among certain Angiosperms, in which the strobili are much reduced. We are thus forced to conclude in favour of the first alternative outlined on p. 491, that the Gnetales were at one time a much diversified class of plants.

The recognition of the fact that the Gnetalean fructifications may be interpreted as reduced anthostrobili affords one clue to the origin of the group. Another is to be found in the obvious relationship which exists between these plants and the Angiosperms. We have shown, in the historical sketch, that this affinity has been recognized from early times, and on many hands, though doubt and difference of opinion has existed as to whether these plants should not be included among the Dicotyledons, or even whether the Angiosperms were not derived from them. We have emphasized certain features connected with the fructifications, which strongly support the idea of the existence of a blood-relationship with the Angiosperms. There are, in addition, others of a vegetative nature, such as the very dicotyledonous-like foliage of *Gnetum*, and the mode

of branching in *Gnetum* and *Ephedra*. The dicotyledonous embryo, the general morphology of the seedlings and leaf-trace system, and the presence of elements in the xylem, which more or less resemble true vessels, afford additional evidence.

The question is, then, not as to the existence of an affinity, but the degree of that affinity. The possibility of including the Gnetales within the Angiosperms is, as is now generally agreed, ruled out of court by the increasing importance, which has continued to be attached, since the days of Robert Brown, to the distinction between naked and enclosed ovules.

Nor could the present day Gnetales have been the ancestors of the Angiosperms, for not only are they contemporaneous with them in point of time, but their fructification *remains* a pro-anthostrobilus, as opposed to the eu-anthostrobilus or flower of the Angiosperms.

On the other hand, all the evidence points to the conclusion that these two groups have sprung from a common ancestor, the fructification of which was also a pro-anthostrobilus, and that they have progressed in a great measure parallel to one another. This hypothetical ancestor we have already attempted to restore provisionally in our earlier paper, under the name of the Hemiangiosperm.[1] It possessed a typical pro-anthostrobilus, which was amphisporangiate, and also a perianth. In the male fructification of *Welwitschia*, all the organs of this strobilus are still present, though in a much reduced form. The advance towards the monosporangiate condition has, however, been great, for the megasporangium, though present, has ceased to function. The female of the same plant, and both the male and female fructifications of *Gnetum* and *Ephedra*, are easily derived by the further reduction of such a strobilus.

Though the male strobilus of *Welwitschia* may be regarded as having retained a larger number of primitive features than the other fructifications, it is no doubt itself extremely reduced, and thus differs very widely from the typical pro-anthostrobilus of the Hemiangiosperm. While we can still trace the relationship, so far as the essential characters of the anthostrobilus are concerned, it is not easy to restore the exact stages, which represent the path along which the reduction has been carried. Yet, as we have pointed out, the origin of the male synangia of the Gnetales does not present any great difficulty, except in details. The absence of the megasporophyll can be accounted for by the fact that, where great reduction has been the rule, it is not surprising that the total suppression of an unimportant organ should have taken place, since the method of pollen-collection in both the Hemiangiosperms and the Gnetales was, and is, independent of it.

In other respects the scanty evidence to be drawn from a few, such highly reduced strobili compels us to leave the picture unfinished, in the

[1] Arber and Parkin ('07), p. 62, and Fig. 4 on p. 63.

absence of any fossil evidence. The difficulties that beset us here may be illustrated by imagining that we wished to restore the flower of the Ranales, as typical of Dicotyledons, and that only three genera of the Incompletae, such as *Casuarina*, *Populus*, and *Peperomia*, now existed as the survivors of the group.

Further, even if the Gnetales, using the term in a wide sense to designate a once numerous, and now almost extinct, group, preceded the Angiosperms in point of time, as indeed they may have done, it is impossible to derive the Angiosperms from them. For, as we have pointed out, the evidence is all in favour of two lines of evolution, which were not identical but parallel. From the Hemiangiosperms sprang two distinct lines, the primitive Angiosperms (*Ranalian plexus*) and the primitive Gnetales, the three highly-evolved living genera of the latter being their sole survivors. On our view then these primitive Gnetales did not give rise to the Angiosperms, neither did they continue to be in all essentials Hemiangiosperms. Though, as we have pointed out, it is almost impossible to restore these primitive Gnetales, from the scanty evidence at our command, we see no reason to believe that they did not possess as marked peculiarities of their own, as did the primitive Angiosperms. Perhaps the tendency to suppression of the megasporophylls, and to reduction in the organs of the strobilus, may have even then been at work, though in a less marked degree.

General Conclusions.

The systematic position of the Gnetales, and especially their relationship to the Angiosperms, is a question of the greatest difficulty on account of the scarcity of the evidence. Three living genera, two of which are very highly specialized to particular environments, alone exist, and further the group is at present unknown in the fossil state. Yet we are forced to the conclusion that the Gnetales are not a modern group, just springing into being. Like the Angiosperms, they have had a history in past geological periods, though at present we are unable to read the records of their former greatness. This is in the main due to the unfortunate position of our knowledge of the real nature and affinities of the fossil plants occurring in the Cretaceous and Tertiary rocks.

The three survivals of this ancient group possess fructifications which we interpret as reduced pro-anthostrobili. We find that the same principles of evolution, which we have already applied to unravel the tangled skein of Angiospermous descent, are equally applicable to the Gnetales. It is thus to the credit of the Strobilus Theory, as a working hypothesis, that while the Gnetales themselves throw but little light on the ancestry of the Angiosperms—a conclusion which we have consistently maintained—the application of the theory, founded on Angiosperm evidence, to the elucidation

of the Gnetales, does furnish us with a logical argument to account for the very existence of these plants at the present time.

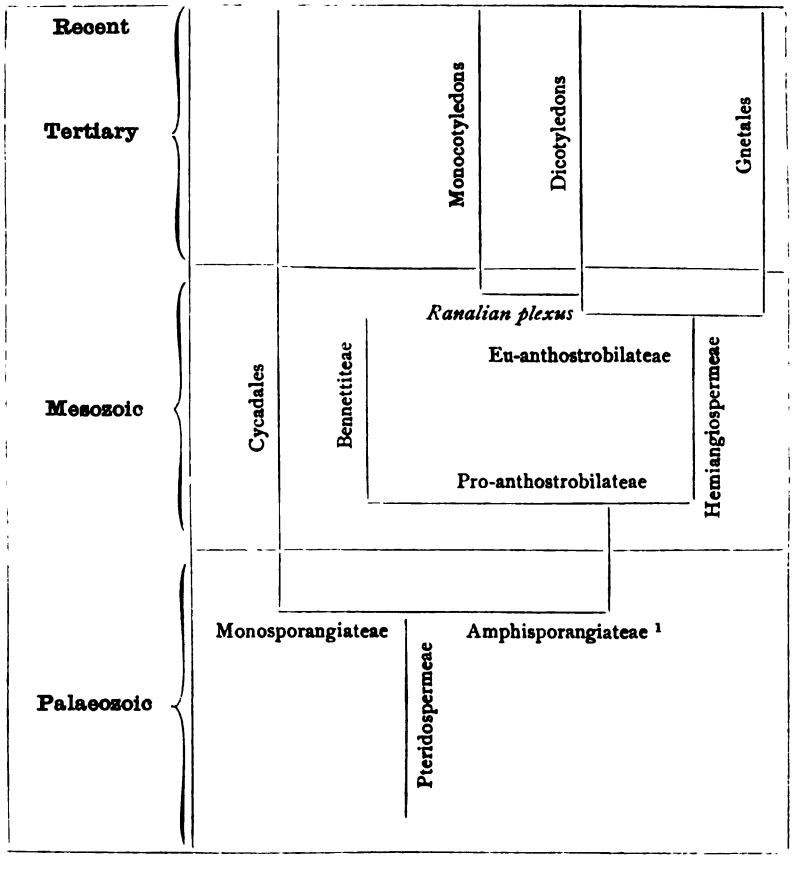
Of the three genera, *Welwitschia* appears to us to be the most primitive as regards its fructification. We have shown that the male strobilus of this plant is, in its essential features, an amphisporangiate Pro-anthostrobilus. It possesses a perianth, and both male and female organs, the latter occupying the apical portion of the strobilus. The female fructification of the same plant, and both the male and female fructifications of *Ephedra* and *Gnetum*, we regard as pro-anthostrobili reduced to the monosporangiate condition, each possessing organs homologous with those of the amphisporangiate male strobilus of *Welwitschia*.

The reduced strobili of the Gnetales are grouped in dense aggregates or clusters, which in the case of *Ephedra* and *Welwitschia* find a close parallel among the Amentiferae. The parallelism with *Populus*, especially with the amphisporangiate species *Populus glauca*, Haines, is very striking. These two genera might almost be spoken of as the Amentiferae of the Gnetales.

We regard the Gnetales as a race of Gymnosperms, nearly related to the Angiosperms. There are many and varied indications that both groups have sprung from a common stock, and that their lines of development have, in many respects, continued parallel. The common ancestors of the Angiosperms and Gnetales are the Hemiangiosperms, a race as yet hypothetical.

The following table expresses the relationships of the Gnetales to those groups to which, on our view, they are most nearly related.

TABLE OF ANGIOSPERMOUS AND GNETALEAN RELATIONSHIPS.



We would here express our thanks to Miss Agnes Robertson, D.Sc., for kindly assisting us in certain matters in connexion with these studies, and for drawing one of the figures.

[1] By a printer's error, the word 'Diplosporangiateae' appeared here in the corresponding table on p. 77 of our earlier paper.

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On the Real Nature of the Tracheae in the Ferns.

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With Plate XXVIII.

THE following observations were originated by an investigation recently carried on by Dr. Kidston and myself into the structure of certain fossil Osmundaceae.[1] In some of the specimens that were examined the preservation of the xylem was unusually good, and much information was obtained concerning the detailed structure of the tracheal walls. It was found, however, that our observations on the fossils could not be brought into agreement with the prevalent idea of the structure of a Fern tracheide. This necessitated a re-examination of the xylem of some of the recent Ferns in order to see if this difficulty could not be explained away, eventually leading to the following unexpected conclusions :—

In the first place, it may be stated that the metaxylem of the Filicales is generally regarded as consisting, for the most part, of typical scalariform tracheides ; the typical scalariform tracheide possessing a single series of pits on each of its sides or facets. Very few exceptions to this statement have as yet been recorded. The 'tracheide' type of element is known to be departed from in two cases only : *Pteris aquilina* and *Nephrodium filix-mas* (in the root). In these it is stated that true perforations occur in the end walls of the tracheae, which must, therefore, be regarded as vessels.[2] As regards the kind of pitting, it is known that more than one series of pits occur constantly on the same facet in the xylem elements of *Botrychium*,[3] *Helminthostachys*,[4] and in certain Botryopterideae.

[1] Kidston and Gwynne-Vaughan, 'On the Fossil Osmundaceae,' parts i and ii, Trans. Roy. Soc. Edin., vol. xlv, part iii, p. 759, 1907, and vol. xvi, part ii, 1908.

[2] Russow, 'Vergleichende Untersuchungen,' Mém. de l'Acad. Imp. des Sc. de Saint-Pétersb., Bd. xix, p. 103, 1872.

[3] Idem, p. 118.

[4] Farmer and Freeman, 'Affinities of *Helminthostachys zeylanica*,' Annals of Botany, vol. xiii, p. 433, 1899.

It appears from our observations that the above statement is thoroughly misleading, and that a return must be made to the views held by the earlier anatomists,[1] who believed that the xylem elements of the Ferns in general were true vessels and not tracheides at all.

The xylem of the Osmundaceae will first be described, and in particular that of *Osmunda cinnamomea*, in which plant the point in question was the most thoroughly investigated. To begin with, it appears that the Osmundaceae as a whole must be added to the list of Ferns whose tracheae constantly possess more than one vertical series of pits on each side of their walls. Almost all the sides or facets have two or more series of pits (Pl. XXVIII, Fig. 1); indeed, the typical scalariform marking with a single series of pits is only to be found on the smallest elements at the external periphery of the xylem ring and on those of the decurrent protoxylem strands of the leaf-trace. In the fossil representatives of the order the multiseriate nature of the pitting is even more pronounced, walls being often found with as many as four or five regular series. In the largest elements the pits are so numerous, and so irregularly arranged, that in surface-view the walls present the same reticulate or porose appearance as do the xylem elements of *Botryopteris* (Fig. 2).

To turn now to the actual nature of the pits themselves; it soon became clear that there is no pit-membrane whatever separating the cavities of two contiguous tracheae from one another. The pits are, in fact, actual perforations permitting free lateral passage through the side walls of the tracheae. This is not all, for the repeatedly unsuccessful attempts to demonstrate a pit-membrane gradually brought to light the surprising fact that there was no 'middle substance' in the other parts of the wall; not even between the bars of thickening that separate the pits. For instance, in a wall bearing a single series of pits the bars of thickening stretch across quite freely from one corner to another without coming into contact in the median plane of the wall (Fig. 3). The bars are in opposite pairs, one belonging to each of the two contiguous tracheae, and there is an empty space between them. It follows, therefore, that there is a free passage both up and down in the middle of the common wall from one pit cavity to the other throughout the whole series of pits. When there is more than one series of pits on the same side of the wall, matters are not quite so simple, for the parts of the wall separating the several series of pits vertically are solid right through (Fig. 4). At these points the bars belonging to the two tracheae are firmly cemented together by an intervening 'middle substance.' In this case, therefore, although there is the same free passage up and down in the middle of the wall for each series of pits, there is no direct lateral passage from one vertical series to the other.

[1] Dippel, Das Mikroskop (new ed.), p. 280 (1st ed. 1869); Sachs, Textbook of Botany (English ed.), pp. 25-26; Gustav A. Weiss, Anatomie der Pflanzen, p. 271, 1878.

I was able to satisfy myself that these holes are real and normal and are not due to any accident or preservation, because they can be observed in sections taken from fresh and living material. They are, of course, exceedingly narrow, but at the same time they are distinctly visible under a Zeiss D objective in stained sections of sufficient thinness.

The actual formation of these holes can be followed in sections cut at different levels just below the meristem of the apex, in a region where the xylem is still more or less imperfectly lignified. The sections should be treated with some dye that has an affinity for the pectic substances of which the young cell-walls are chiefly composed. The best results were obtained with ruthenium red; a dye especially recommended for this purpose by Mangin [1] and used with success by Allen in his work on the middle lamella.[2] It is made up in watery solution; about ·02 gr. of the dye to 100 c.c. of water. The solution should then be rendered alkaline by the addition of about ·5 c.c. of concentrated ammonia. It should be kept in a blackened bottle, for it is gradually decomposed by the action of light. In any case, its action becomes much less intense if kept for over a month. Sections stained by ruthenium red can be brought up into Canada balsam. Allen states that the stain gradually fades away, but after several months my preparations hardly show any deterioration. A very pretty effect may be obtained by counterstaining with methylene blue, which has an affinity for lignin. Partly lignified walls show gradations in the intensity of the blue stain. Pectic substances also stain well with methanil violet and Hoffman's violet.

When a transverse section of a partially differentiated xylem strand is treated with ruthenium red, the primary walls of the youngest tracheae are stained a deep red. The dye is taken up by the whole thickness of the wall, but at the same time an extremely thin middle lamella is just perceptible as a very fine line more deeply stained than the rest. The primary wall remains comparatively thin except at the angles, which later on become especially thickened (Fig. 5). These angular thickenings stain somewhat differently to the rest of the wall, although they still give a pectic reaction. In a slightly older wall the thick secondary layers will have been deposited on the thin primary wall, over all its surface except those regions destined to become pits. When first deposited these secondary layers also consist of pectic substances and take up the stain almost as readily as do the primary walls (Figs. 6 and 7). At this stage a fair amount of protoplasm is still present in the young trachea, but the nucleus seems to disappear during the deposition of the secondary layers or even earlier. Longitudinal

[1] Mangin, 'Sur l'emploi du rouge de ruthénium en anatomie végétale,' Comptes-Rendus de l'Acad. des Sc. Nat. de France, t. 116, p. 653, 1893.

[2] Allen, 'On the Origin and Structure of the Middle Lamella,' Bot. Gaz., vol. xxxii, No. 1, p. 1, 1901.

sections indicate that the tracheae undergo a considerable amount of sliding growth which must take place while they are still in a thin-walled condition. The first signs of lignification in the secondary layers appear at the corners. Shortly afterwards they become lignified all over, and so do the angular thickenings of the primary walls (Figs. 6 and 7, unshaded), and also the regions separating the vertical series of pits. All the rest of the primary wall remains pectose, and as soon as the lignification of the above-mentioned regions is completed it begins to show signs of disintegration. Its substance breaks up and becomes granular and then gradually disappears without, however, altering its composition, for to the last it is coloured by the pectic stains (Fig. 8). Its complete re-absorption leaves an empty space between the two bars of secondary thickening, or, in the region of a pit, sets the cavities of the two contiguous tracheae into continuity. The pectose portions of the primary wall seem to be reabsorbed more rapidly in some specimens than in others, and in all cases some remains are still to be observed coating the extremities of the persistent lignified parts (Figs. 3 and 4). Where a trachea borders upon a cell of parenchyma the primary wall of the trachea remains intact, and so also does the middle lamella between it and the wall of the parenchymatous cell. It follows from this description that the holes in the tracheal walls are due to the re-absorption of *the whole thickness of the primary wall* of the young tracheae at the points in question, and not merely to the disappearance of the middle lamella.

Longitudinal sections are not particularly helpful in demonstrating these holes. To obtain an ideal section the razor should pass twice through the same series of pits and then the several bars, being loose and unconnected, all fall away from one another. In thicker sections the pectic remains that coat the ends of the persistent portions of the primary wall are visible through the unstained lignified parts and simulate a continuous middle substance. If, however, the cut edge of a vertical wall be brought into focus it is seen that the pits are extremely narrow, and that the bars of thickening often vary greatly in width. They usually project much further into the cavity of the element in the neighbourhood of the corners than elsewhere. This accounts for the overlapping outlines so often seen in transverse section (Fig. 8).

The same type of element was found in stem, leaf, and root of all the species of *Osmunda* and *Todea* that were examined, and it may also be confidently inferred in the fossil representatives of the order. A reference to Fig. 11, which is a photograph of the xylem of *Osmundites skidegatensis* will illustrate the peculiar and at first inexplicable appearance of the tracheal walls that initiated these observations. It now appears obvious that the black bars represent the bars of thickening, while the white spaces represent holes that occur in the substance of the walls. In the other fossil members of the order the xylem-walls vary very much in appearance

according to the nature of the preservation, but in all cases they may be readily brought into agreement with the type of element described above, except the largest tracheae, which possess irregularly scattered more or less rounded pits. In these it is probable that each pit is an isolated perforation whose cavity does not communicate with those of the others.

It is no longer possible, therefore, to regard the xylem elements of the Osmundaceae as tracheides; on the contrary, they are a special and very peculiar type of vessel. In each separate xylem mass of the stele there is a perfectly free passage for water in all directions from one element to another both vertically and horizontally; to say nothing of the passages that occur in the very substance of the walls.

In order to obtain some real genuine tracheides for the purpose of checking the observations made upon the xylem of the Osmundaceae, I next examined that of *Nephrodium Filix-mas*. In this plant the xylem elements are typically scalariform, with one series of pits only on each side of the wall. But here also it was found that the primary wall is completely re-absorbed both in the region of the pits and between the bars of secondary thickening (Fig. 9). In fact, they have no right whatever to be called tracheides, but are vessels of the same type as those of the Osmundaceae, the only difference being that they are typically scalariform.

Pteris aquilina was next tried, and here a somewhat different type of element was met with, although still undoubtedly a vessel and not a tracheide. They are typically scalariform, and here again the pits, both on the end walls and on the side walls, are true perforations. They differ, however, from the vessels of the Osmundaceae and of *Nephrodium*, in that those parts of the primary wall that connect the opposite transverse bars of secondary thickening in the middle of the wall are here maintained intact even at maturity. The bars of each pair are, therefore, joined together by a cementing substance across the thickness of the wall. These persistent parts of the primary wall do not become lignified, but remain pectic in character and stain readily with ruthenium red. When a tracheal wall treated with this reagent is regarded in surface view, the pectic middle substance will appear as a red area shining through the lignified and unstained secondary layers and outlining the inner limits of the pit cavities as shown in Fig. 10. The shaded regions represent the pectic cementing substance connecting the bars in the middle of the wall, and it is seen that the widest extension of the pits greatly exceeds their apertures into the lumen of the vessel.

Sachs in 1874 was already aware that the xylem elements of *Pteris aquilina* bore true perforations on their lateral walls. The figures he gives in his textbook (l.c. Fig. 27) illustrate most clearly and accurately the actual state of affairs. On the other hand, they escaped the notice of De Bary, Strasburger, and subsequent observers. Very probably this was

due to a natural tendency to cut as near to the apex as possible in order to avoid the inconvenience caused by the mature sclerenchyma. The tracheae in such regions would be too young for the complete disappearance of the primary wall.

Vessels of the *Pteris* type seem to be very widely distributed among the Pteridophyta. They were observed in the Polypodiaceae, Cyatheaceae, Hymenophyllaceae, Gleicheniaceae, Schizeaceae, Marattiaceae, Ophioglossaceae, Lycopodiaceae, and in the fossils *Psaronius*, *Botryopteris*, and *Zygopteris*. With regard to *Zygopteris*, Professor Weiss has pointed out to me that the presence of open passages in the side walls of the tracheae renders it easy to account for the thyloses he found far away from any living elements in the most central tracheae of the non-parenchymatous xylem mass.[1]

As a matter of fact, true and indisputably imperforate tracheides were not met with in any one of the Pteridophyta examined. These were, however, far too few to permit any generalization. On the contrary, true tracheides must occur in the Calamites at any rate, for it appears that vertical striations can be observed on their pit-membranes.

Even in the Gymnosperms the true tracheide is by no means so general a type as might be supposed. From some preliminary preparations made by Miss S. Greves that I have been permitted to examine, it appears that true perforations occur in the longitudinal walls both in the primary and secondary xylem of some of the Cycads. This is probably the case also in the secondary wood of *Araucaria*; at any rate, it has not been found possible to demonstrate a pit-membrane there.

Considered from a mechanical point of view, elements of the Osmundaceous type would appear to be very unsuitable for conferring strength and rigidity to the xylem as a whole. The fact that in the Ferns as a whole, and particularly in the Osmundaceae, the function of support is taken over almost entirely by masses of strong sclerenchyma in the ground tissue, has perhaps rendered the existence of this type of vessel possible. Even under these conditions it seems that mechanical exigencies prevent the width of the tracheal wall exceeding a certain limit. In the Osmundaceae the limit of width for a wall with a single series of pits is about 45 μ. Walls of greater width have the bars cemented together about half-way across, so that there are two series of pits, and may attain as much as 70 μ. With three series of pits they reach 90 μ, and with four up to 104 μ. In *Nephrodium Filix-mas*, walls with a single series were found as much as 55 μ wide. Of course, when each pair of bars is cemented together along the whole width of the wall, as in *Pteris aquilina*, the mechanical efficiency is greatly increased and walls with a single series of pits may be as wide as 130 μ.

[1] F. E. Weiss, 'On the Thyloses of *Rachiopteris corrugata*,' New Phyt., vol. iv, No. 4. p. 82, 1906.

As regards the pattern of the pitting, I am inclined to regard rounded pits as more primitive than scalariform. The rounded pits would be at first irregularly distributed, and their subsequent arrangement in regular vertical series would prepare the way for an advance towards the multiseriate vessel of the Osmundaceous type. The typical scalariform pattern may have been derived from the multiseriate type, but not necessarily so. It may equally well have arisen by the approximation and transverse elongation of a single series of rounded pits. A single series of more or less rounded and very irregularly scattered pits is still to be met with in the xylem of *Botrychium* and *Helminthostachys*.

SUMMARY.

The xylem elements of the Pteridophyta are, for the most part, vessels with true perforations in their longitudinal as well as in their terminal walls.

In the Osmundaceae, *Nephrodium Filix-mas*, and probably others, a special type of vessel occurs which is characterized by the complete disappearance of the primary tracheal wall at certain points, so that the cavities of the pits are vertically continuous in the middle of the wall.

It is probable that more or less rounded pits preceded the transversely elongated pits of the scalariform type in the Filicales.

DESCRIPTION OF FIGURES IN PLATE XXVIII.

Illustrating Mr. Gwynne-Vaughan's paper on Tracheae in the Ferns.

Figs. 1, 2, and 11 are photographs, the rest are drawings.

Fig. 1. *Osmunda cinnamomea.* Wall of vessel with three regular series of pits in surface view.

Fig. 2. *Zalesskya gracilis.* Wall of vessel with porose pitting in surface view.

Fig. 3. *Osmunda cinnamomea.* Transverse section of a wall with a single series of pits.

Fig. 4. *Osmunda cinnamomea.* Transverse section of a wall with three series of pits.

Fig. 5. *Osmunda cinnamomea.* Transverse section of some very young xylem elements. The primary wall alone is formed and it is especially thick at the corners.

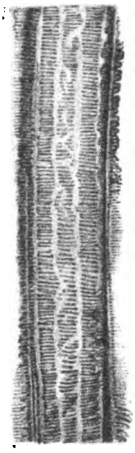
Figs. 6 and 7. *Osmunda cinnamomea.* Transverse section of some older xylem elements. The layers of secondary thickening have been deposited on the pectose primary wall. In some the secondary layers are lignified (lightly shaded), in others they are still partly pectose (darker shading) and protoplasm is still present. Note that the lignification commences at the corners, and also that the primary wall also lignifies at these points.

Fig. 8. *Osmunda cinnamomea.* Transverse section of still older elements. All the secondary elements are now lignified, and so are the corner-pieces of the primary wall. Other regions of the primary wall are being re-absorbed, leaving empty spaces in the middle of the wall.

Fig. 9. *Nephrodium Filix-mas.* Transverse section of the mature xylem. The walls only bear one series of pits. The cells on the left are xylem parenchyma.

Fig. 10. *Pteris aquilina.* Wall of a vessel in surface view. The shaded regions indicate the parts of the pectose primary wall that persist and cement the bars together.

Fig. 11. *Osmundites skidegatensis.* Transverse section of the xylem.



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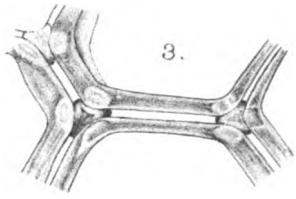

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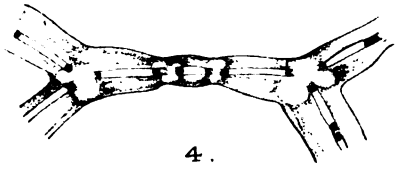

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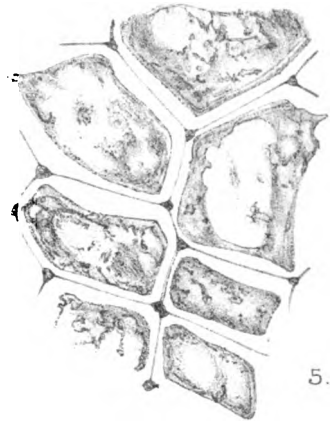

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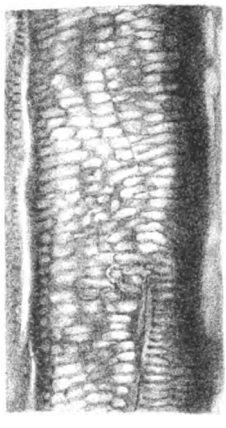

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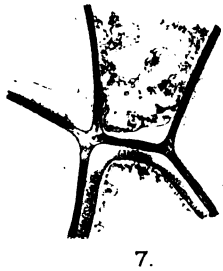

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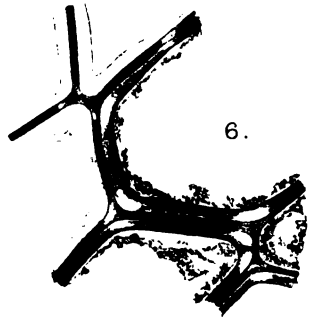

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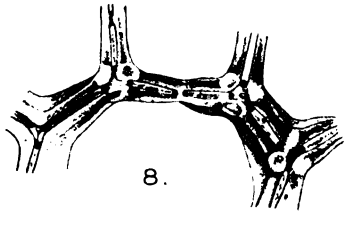

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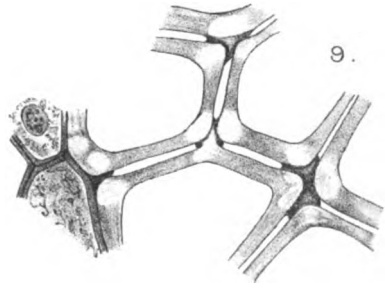

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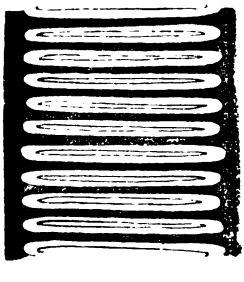

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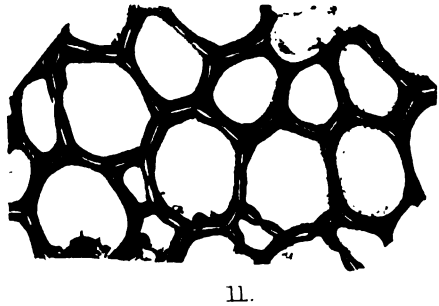

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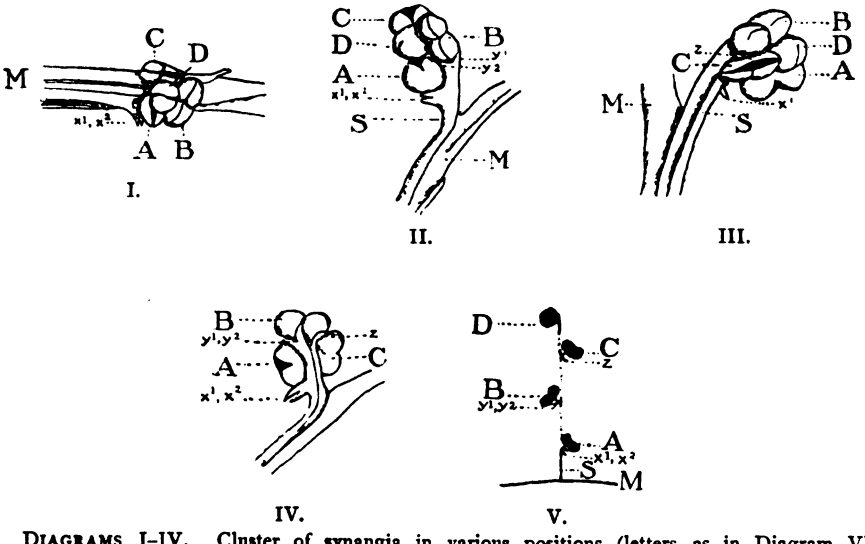
GWYNNE-VAUGHAN — TRACHEÆ IN FERNS.

NOTES.

NOTE ON AN ABNORMALITY FOUND IN PSILOTUM TRIQUETRUM. —The following note is a description of an unusual structure remarked by Dr. Benson in a dried plant of *Psilotum triquetrum*, sent her from New South Wales. My thanks are due to Dr. Benson for kindly allowing me to describe and figure this structure.

Some distance from the apex of the plant there occurred, among the ordinary synangia, a cluster of four synangia borne together on a common stalk. Three of these synangia were bilocular, while the fourth was unilocular. Diagrams I, II, III, IV, represent front, side, and back views of the cluster, and show the four synangia (A, B, C, D) with two forked bracts (x^1, x^2, y^1, y^2) and one single bract (z).



DIAGRAMS I–IV. Cluster of synangia in various positions (letters as in Diagram V). DIAGRAM V. M = main axis; S = common stalk, bearing cluster of synangia, the dotted portions represent the internodes, elongated for the purposes of the diagram; A, B, C, D = synangia; x, y, z = bracts.

On dissection it was found that no further bracts were present. The common stalk was flattened and was about a quarter of an inch in length. It bore, first, a bilocular synangium (A) with a double bract (x^1, x^2); secondly, a similar synangium with its double bract (B, with y^1, y^2); thirdly, a bilocular synangium (C) with a single bract (z); and finally it was terminated by a unilocular synangium (D) destitute of bracts (see Diagram V). Each of the first three synangia with their bracts was almost sessile, but the terminal synangium occurred at the end of a comparatively elongated, and also much curved, portion of the common stalk.

This abnormality appears to be of considerable interest in connexion with recent discussions[1] concerning the morphology of the synangium-bearing organ in the Psilotaceae. There seem to be two possible views as to the nature of the stalked cluster of synangia. It may be looked upon as equivalent to one of the normal aerial branches of *Psilotum*, which has for some reason become abortive in development; on the other hand, it may be regarded as derived from a single proliferated synangium-bearing appendage. The common stalk, arising from the main axis, and bearing the cluster of synangia, affords a striking point of difference from the ordinary sessile 'sporophyll,' but, since in the nearly allied *Tmesipteris* the synangium-bearing organs are stalked, this difficulty does not seem insurmountable. The presence of a terminal synangium is of extraordinary interest, and has not, so far as I am aware, been yet described in *Psilotum* (cf. *Tmesipteris*[2]). The theory that the abnormality is equivalent to a single 'sporophyll' appears to me far the more probable, especially when considered in the light of similar abnormalities in *Tmesipteris*, in which genus ordinary branches do not occur. It is not easy to suppose such a structure as this cluster to be of a foliar nature, for it can hardly be described as a repeatedly dichotomizing leaf.[3] It is more possible to look upon it as a proliferated sporangiophore (primitively non-foliar in nature[4]), bearing several synangia, associated with several bracts, and terminated by a synangium at its apex.

M. G. SYKES.

ROYAL HOLLOWAY COLLEGE, LONDON.

INTERNAL PHLOEM IN MYRISTICA.—In *M. fragrans*, Houtt., the midrib of the lamina of the leaf, which is a direct continuation of the petiole, contains a vascular cylinder which is much greater in diameter in the tangential than in the radial direction; its dorsal half is arc-shaped, while the ventral half is flat, the xylem of the latter is incomplete, two of the phloem-groups composing it being without any xylem, while what xylem there is present is much less thick than that belonging to the dorsal half of the cylinder. Four angular groups of *medullary phloem* occur, one of which is in direct connexion with one of the large phloem-groups of the ventral half of the cylinder which has lost its xylem. In the petiole these medullary phloem-groups at once tend to pass into the broad medullary rays, which they entirely fill, but one group is still left in the pith. At the base of the petiole the cylinder becomes, by the passing across of its ventral bundles, an arc of three arched bundles, each of which has a medullary phloem-group bending round each of its ends, thus standing half-way between pith and normal phloem. Thus, *before the leaf-bundles enter the stem, the medullary phloem leaves the pith and unites with the ordinary phloem of the cylinder.*

In *M. glaucescens*, Jack., essentially the same structure occurs.

In the *peduncle* of the fruit of the first-named species it was very interesting to find, what one might, in an organ of this nature, *a priori* expect, a transitional struc-

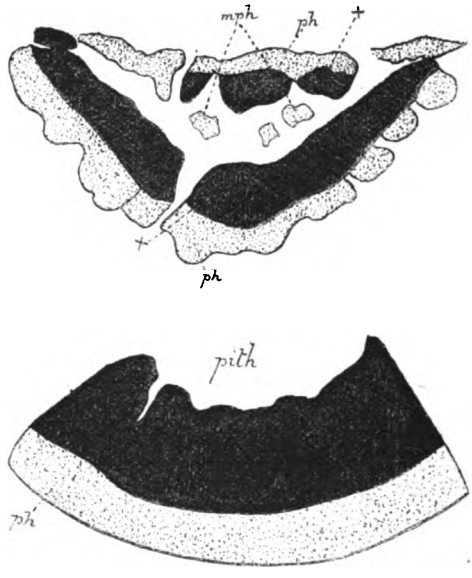
[1] Scott, D. H., Proc. Roy. Soc., 1897, and Prog. rei. bot., 1907; Thomas, A. P. W., Proc. Roy. Soc., 1902; Sykes, M. G., Ann. of Bot., January, 1908.

[2] Sykes, M. G., l. c. Text-fig. vii, x.

[3] Cf. Abnormalities in *Psilotum*, referred to by Thomas, l. c., p. 349.

[4] Sykes, l. c., p. 82.

ture between that of the leaf and that of the vegetative *stem*, *which latter is entirely devoid of medullary phloem*. There was no phloem in the middle of the pith, as is the case in the leaf, but at the *inner* angle of one of the bundles, just at the entrance to a medullary ray, I observed a small isolated group of phloem, and I also noticed a large group of phloem in two or three of the medullary rays which was in direct connexion with, and a continuation of, the ordinary phloem of the cylinder. Both of



Myristica fragrans, Houtt.

FIG. 1. Transverse section of vascular cylinder of midrib of leaf-blade showing the groups of medullary phloem (*mph*). +, xylem; *ph*, normal phloem.

FIG. 2. Transverse section of segment of vascular cylinder of vegetative stem showing complete absence of medullary phloem.

(Both Figs. diagrammatic and × 75.)

these appear to me to represent fixed stages in the passage of the medullary phloem from the pith outwards to the external phloem of the cylinder.

Holding the view which I do that the present-day structure of the leaf reveals to us in so many cases what was the structure of the *stem* in the not very remote ancestors of the plant, I draw the conclusion that *Myristica* once had *internal* phloem in the stem, but has now lost it, and I go further and say that this medullary phloem of the leaf of to-day represents a vestige of a system of complete medullary bundles which were characteristic of both stem and leaf.

The peduncle, an organ which I have found in so many other cases to retain a primitive structure which has vanished from the vegetative stem, has in this genus *partially* retained the primitive character of medullary phloem, of which there is no trace remaining in the vegetative axis.

W. C. WORSDELL.

KEW.

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ANNALS OF BOTANY

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ASSISTED BY OTHER BOTANISTS

London

HENRY FROWDE, AMEN CORNER, E.C.

Oxford

CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1908

Printed by HORACE HART *at the* CLARENDON PRESS, *Oxford.*

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The Behaviour of the Chromosomes in Pinus and Thuja.

BY

I. M. LEWIS,

University of Indiana.

With Plates XXIX and XXX.

ALTHOUGH the behaviour of the chromosomes in the two divisions attending spore formation in the higher seed plants has been a favourite subject for research, the majority of investigators have confined their attention to certain favourable forms of the Angiosperms, and only a few extensive investigations have been made in the Gymnosperm group. Among these, the work of Miss Ferguson ('04) for *Pinus* is doubtless the most comprehensive. The results obtained by that writer differ so much from conditions that are known to exist in other forms that their confirmation seems much to be desired. Certain other questions have arisen since the time at which the genus *Pinus* was investigated which render desirable a study of some members of this group. Among these questions may be mentioned the manner of conjugation of the maternal and paternal chromosomes to form the bivalents and the possibility of recognizing prochromosomes in the resting nucleus.

The forms investigated were *Pinus strobus*, *P. austriaca*, *P. Laricio*, and *Thuja occidentalis*. Figures are shown from the different species, but when differences exist such differences have been noted. Frequent references are made to the work of Miss Ferguson ('04) which is, in a large measure, confirmed by my own results.

METHODS.

The methods used were such as are employed in any piece of modern plant cytology. The cones were fixed as soon after their removal from the tree as was possible. The fixing fluid used was the chrom-osmic-acetic fluid of the formula proposed by Mottier ('97). The staminate cones were cut into small pieces, and the hard points shaved off with a razor, thus rendering the sectioning of the pieces more satisfactory. In some cases the individual sporophylls were removed and fixed. This causes considerable difficulty in sectioning, but the fixation in material so treated was found to

[Annals of Botany, Vol. XXII. No. LXXXVIII. October, 1908.]

be perfect. The time of fixation was from twenty-four to thirty-six hours, and the material was then washed in running water twelve hours, passed through the grades of alcohol, chloroform, and paraffin, and embedded in paraffin. Sections were prepared varying in thickness from five to twenty microns. Bleaching was effected by placing the slides for a few minutes in an alcoholic solution of hydrogen peroxide. The stains used were the triple stain and Haidenhaine's iron-alum haematoxylin. For the stage of the resting nucleus and mitotic figures the triple stain is far superior, but for other stages of the prophase the iron-alum seems to give better results. Full sets of preparations were made using both stains.

The Phenomena of the Prophase.

The present trend of thought regarding reduction has directed attention especially to the prophase of the first division, with especial emphasis upon the resting nucleus and the stage of synapsis. At the close of the last division in the archesporial cells of both *Pinus* and *Thuja* the nucleus soon passes over into what is universally regarded as the resting stage. The nuclear content consists at this time of irregular chromatic lumps connected by delicate, anastomosing linin threads which stand out with great clearness and beauty in sections well differentiated with the triple stain. Concerning the question of whether two substances are to be distinguished here, nothing new can be added, but there is no doubt that the staining reaction of the chromomeres and the so-called linin is quite distinct. There seems to be a tendency on the part of the chromatin to become quite finely dissected, but there are always some bodies which remain quite large (Pl. XXIX, Fig. 1). The number of these lumps is always quite in excess of the number typical of the somatic chromosomes of the sporophyte generation and does not approximate to this number by subsequent fusions. Mottier ('07) has shown for *Lilium* that the extent of dissection in the chromatic masses may be quite variable in closely connected cells of the same antherloculus. In this genus it seems that in some of the cells the bodies in question become finely broken up and separated along the linin threads, while in others they remain more closely intact. He has raised the question as to what extent this difference in appearance may be caused by the reagents, as it seems quite probable that the action of osmic acid might exert some such influence on the chromatic substances, but no very definite results have as yet been obtained. This writer observed and figured nuclei exhibiting both of these conditions as they passed on into the synaptic condition, and they seem to behave in precisely the same manner, no matter which condition prevails in the resting nucleus. He concludes, therefore, from a long comparative study of the forms investigated, that the number and size of the chromatic lumps is a matter of minor importance. Miyake ('05) made a comparative study of a long list of plants, and he finds that

the lumps are quite variable in respect to both size and number. In *Lilium* he states that the number is often greater than that of the somatic chromosomes, and does not reach this number by subsequent fusions. In other genera he has, however, reported conditions which are more in harmony with the idea of prochromosomes. Overton ('05) states that in *Thalictrum purpurascens, Helleborus foetidus*, and *Campanula grandis*, the chromosomes of the last somatic division remain visible in the nucleus of the spore-mother-cell as rather large lumps, which resemble the somatic chromosomes, and which always coincide with them in number. He speaks of these accordingly as 'prochromosomes'. These lumps he finds to be generally arranged in pairs. The linin strands connecting them are always arranged parallel, and some of the chromatin not contained in the bodies is distributed along the strands. Previous to the synaptic phase, it often happens that the prochromosomes become broken up and distributed along the threads of linin, and thus there are formed two fine continuous threads of linin studded throughout with chromatin. In this condition synapsis is reached.

In neither *Pinus* nor *Thuja*, as stated above, does the number of chromatic bodies ever approach the number of somatic chromosomes. These bodies are always quite numerous, but they do not appear to become so finely granular and dissected as seems to be the case in *Lilium*. There is also no tendency toward an approximation in pairs. The linin threads are not arranged parallel to each other, but rather anastomose in all directions. The chromatin is often distributed for some little distance along these strands, but it is confined principally to the chromosomes themselves. It often happens that two or more bodies are found lying together in close proximity, but no good reason is seen for interpreting this as an indication that they are preparing for fusion in pairs. Equally clear is it that an occasional linin thread follows for a short distance the same course as one lying near it (Fig. 1), but, judging from the extreme rarity with which this phenomenon occurs in these genera, it is my opinion that this is merely a matter of chance and has no significance whatever.

Allen, Guignard, Berghs, Cardiff, and others have reported conditions for many genera of plants in which the linin threads become arranged parallel to each other, and then by the reticulation and distribution of the chromatin along these threads two fine spirems are formed prior to synapsis. During the synaptic phase these spirems fuse more or less completely into one. In both *Pinus* and *Thuja* the first indication that the synaptic condition is approaching is the withdrawal of the nuclear network toward one side of the cavity. There is no evident change at this time in the content either in structure or staining reaction. The chromomeres retain approximately the same size as before, and the linin still retains its characteristic staining reaction. The net becomes somewhat contracted towards the side

of the cavity, but a few chromomeres may be seen lingering behind. Linin threads pass out toward the nuclear membrane, and the whole gives one the impression that the content has simply contracted. There is at this time no structure which can be regarded as a continuous spirem (Fig. 2). The contraction continues, the elements retaining the same structure and staining reactions, and in such nuclei there can be no doubt of the manner in which the chromatic content approaches synapsis. There is no indication of a pairing or conjugation of the chromomeres or of the linin threads, but rather a contracting of an irregular reticulum of single threads studded throughout with single chromomeres (Fig. 3). Before the greatest degree of contraction is reached the chromomeres give evidence of dissolution into the smaller granules of which they are composed. These granules are apparently drawn out along the linin strands, numerous cross strands are drawn in, and the reticulum seems to be passing over into a skein or spirem (Fig. 4). The content stains more uniformly now than prior to this time. Following this condition the contraction becomes so dense that even in the best differentiated preparations it is impossible to observe clearly what takes place (Fig. 5). One can only observe the phenomena leading up to this condition and the recovery from it.

Synapsis has been, and still is, variously interpreted by different workers. That it is a perfectly normal step in the process of reduction seems now to be generally agreed, Schaffner ('07) being perhaps the only investigator who still maintains the view once prevalent that this condition is an artifact. It must be admitted that the nucleus is at this time the seat of great activity, that the nuclear cavity is enlarging to its final size, and that conditions are therefore favourable for faulty fixation. But when one considers the differences between the structure of the nuclear content as it passes into this condition and as it recovers from it, together with the uniformity with which it occurs during the reduction division, and the fact that it has been frequently observed in living cells, the last objection seems to fall.

The term synapsis has come to be used in a different sense from that in which it was first employed by Moore ('95). This writer was the first to call attention to the fact that the unilateral massing of the chromatic content of the nucleus is a normal step in mitosis. At that time, although all other investigators held this condition to be purely an artifact, Moore expressed the belief that whatever synapsis might eventually turn out to be, 'it is evidently a cellular metamorphosis of profoundly fundamental character,' and, judged in the light of present interpretations, he could not have made a better supposition. The term synapsis was used by Moore to denote a definite stage or phase of mitosis, the meaning of which was not known and only in a vague way conjectured. Its application to this phase was soon adopted, and all investigators, whether recognizing the stage as normal

or as an artifact, employed this term to denote it. As the meaning of the synaptic phase became clearer it was generally recognized as being concerned with the uniting of somatic univalents in pairs to form the heterotype bivalents. Accordingly the term came to be used by certain observers, as suggested by McClung ('02), to mean 'the fusion of single chromosomes into multiple ones, usually of a bivalent value'. It has been suggested therefore that a new term, synizesis, be adopted to signify the stage of mitosis first denominated as synapsis, and that the latter term be used to denote the conjugation of the maternal and paternal chromosomes. To this there seems to be certain well-founded and serious objections, chief among which may be mentioned the fact that the exact meaning and significance of this stage of mitosis has not been definitely and finally determined. The term has been used through all the literature of the past ten years to denote that very definite stage of mitosis which has been universally observed and which has as yet been only partially explained. The term should be retained with its original meaning whatever significance may be attached to the stage of mitosis it represents. If the meaning of synapsis should be found to be, as is strongly probable, the conjugating of univalent chromosomes, then we would have two terms which signify the same thing and the one would be superfluous, a condition recently reported by Miss King ('07) for *Bufo*.

The tightly contracted stage of synapsis persists frequently for several days, depending upon growth conditions, and in any sporangium the cells reach this stage quite uniformly. Cardiff ('06) has attempted to explain the one-sided position of the chromatin mass as due to gravity, but in neither of the two genera investigated does this explanation hold. In cells lying adjacent to each other, the contraction has been always toward the periphery, but without any apparent relation to the stimulus of gravity. From the balled-up mass threads eventually begin to disentangle themselves and pass out into the cavity. These threads unless cut by the knife are always in the form of loops (Fig. 6).

The spirem thread is now seen to exhibit a very uniform structure. The chromomeres are much more uniform in size than at the beginning of the synaptic phase, and they are embedded in the more lightly staining linin. The thread at this time is seen to show indications of longitudinal fission. This first becomes evident from a careful examination of the chromomeres themselves. In sections well differentiated with the iron-alum stain the chromomeres are clearly seen to occur quite regularly in pairs, although there is an occasional unpaired one (Fig. 6). Allen suggests that the appearance of double chromomeres at this time has been confused, because the chromomeres are not always differentially stained, and, being larger than the linin cord in which they are embedded, the swellings which project over the side have been mistaken for the double rows of chromo-

meres. However, this objection does not hold true for these genera. In sections in which the parts of the spirem are well stained differentially, the chromomeres are clearly observed in pairs, and this pairing is interpreted as due to a longitudinal fission (Figs. 6, 7). The formation of the spirem from the reticulum of the resting nucleus together with its contractions and recovery from synapsis and subsequent fission agrees closely with that given by Miss Ferguson ('04).

In a short time the nuclear thread has completely disentangled itself and wound quite evenly throughout the entire cavity (Fig. 8). This figure is drawn from a section twelve microns thick. The free ends are seen to occur on the cut surfaces, and a study of this stage in preparations cut thick enough to include the entire nucleus has convinced me that there is present at this time an endless spirem. The spirem is quite jagged or granular in appearance, but when one observes closely the chromomeres the double nature is always revealed.

The halves of the spirem now tend to diverge somewhat at intervals, but only in occasional nuclei do they ever become widely separated. The fission of the spirem was preceded by longitudinal fission of the chromomeres, and this was evident at the time the skein loosened up from the synaptic ball. The longitudinal fission never becomes decidedly pronounced in either genus, in fact it might readily be overlooked entirely. Fig. 9 is drawn from the same loculus as Fig. 12. The majority of nuclei exhibit the structure of Fig. 9. These figures are typical of the spirem at this time. It has begun to stain much more uniformly than at the stages immediately following synapsis and the ragged nature is beginning to disappear. In place of such a structure a smooth homogeneous cord is formed (Figs. 10, 11). When one considers the extreme rareness with which nuclei are met with that reveal a divergence of the halves of the spirem, one is strongly inclined to disregard this as a normal step in the process of chromosome development. As the spirem shortens and thickens somewhat it becomes generally quite evenly distributed, and almost all evidence of longitudinal fission is lost.

While these changes have been taking place in the chromatin, certain changes have gone on in the cell itself, which seem to be typical of this stage of mitosis and which may serve in a general way as an index of such stages. The cells during the resting period of the nucleus are packed together closely and are always polygonal in shape. The nucleus occupies a position near the central part of the cell. This position of the nucleus does not persist long, and gradually it tends to move toward one end of the cell. The cells begin to show signs of dissolution from each other by the time the synaptic mass has reached its state of greatest contraction, and when the nucleus has fully recovered the cells have rounded up and the tissue connexion has been lost.

That synapsis has to do with the rearrangement of the chromatin seems probable, but just what takes place is a matter of pure theory. Farmer ('07) suggests that there probably occurs in synapsis an organization of the homologous chromomeres into chromosomes ; that the homologous chromosomes so derived from the maternal and paternal chromatin respectively then unite to form the heterotype bivalents. The conjugation of the somatics is conceived as being effected end to end. Each bivalent consists, therefore, of homologous maternal and paternal primordia. The question naturally arises here as to just what significance is to be attached to the term homologous ; for it becomes at once evident that, if the chromosomes, so conjugating during synapsis, are exact homologues, there is no basis for the transmission of paternal differences, unless we modify our conception by the idea of dominance. If these members of the bivalents are exact homologues, then the first division is equational and not differential, although reducing.

The extent to which affinity of the primordia will cause them to unite seems to underlie all theory of chromosome formation. For certainly the theory of the individuality of chromosomes, the formation of differentials, and the conjugation of somatics during synapsis, is based on the affinity of the units of which they are composed. That this affinity is based on chemical and physical laws seems quite reasonable, but one must not lose sight of the fact that these activities are taking place in living protoplasm.

Mottier ('07) suggests that during synapsis pangens of like affinity become united into homologous chromomeres, and that these chromomeres then come together to form chromosomes. This idea, carried to its extreme form, must arrive at the conclusion that certain chromosomes are composed always of the same primordia, for if homologous units unite persistently they must form bodies which represent always the same characters, and in this sense they will be individuals. Farmer ('07) maintains that 'the chromosome must be indifferent as to the chromomeres which enter into its formation'. He states, however, that at meiosis the pairing takes place in such a way that like joins with like to form the pseudochromosomes. It becomes evident that if this condition has become fixed the chromosomes represent groups of homologous characters united together by mutual affinity. But, on the other hand, if the chromosomes are formed out of the chromomeres by the organizing power of the cell, as maintained by some writers, then a chromosome represents only an accidental accumulation of primordia and not a permanent entity.

The behaviour of the nucleolus has been so thoroughly discussed in all research on the behaviour of chromatin that it would seem useless to enter into a further discussion of the matter here. I have not, however, been able to recognize such a direct relationship between the chromatin and nucleolus as described by Cardiff ('06) for *Acer*, but am disposed to agree with the view

already quite well established that the nucleolus is concerned in some way with the elaboration of the chromatin.

My study of the nuclei as they approach and recover from synapsis has convinced me that the difference of interpretation prevailing among cytologists at present in regard to the conjugation of the spirems is due to a confusion of the stages. Cardiff's ('06), Figs. 8, 9, 10, 12, 43, 54, 63 are, judging from my own experience with mitotic division, drawn from post-synaptic nuclei, although they are much more diagrammatic than anything I have encountered in any cells yet studied.

Formation of the Chromosomes and their Distribution to the Daughter Nuclei.

The manner in which the chromosomes are formed from the spirem presents the greatest difficulty of any step in the entire process of mitosis in these genera. The origin of the spirem and its behaviour subsequent to synapsis seems to preclude the possibility that the chromosomes represent pieces of the spirem in which the longitudinal fission has again become evident, although such a view was formerly held by those investigators who believed in a double longitudinal fission. This view is also held by those who interpret the fission of the prophase as a conjugation of maternal and paternal spirems side by side. Miss Ferguson ('04) maintained that the spirem, which is formed as described above, segments into pieces which equal the number of somatic chromosomes typical of the species. These segments then unite in such a way as to give rise to a sort of reticulum, which finally segments again to form the bivalent chromosomes. Each bivalent consists therefore of two somatics which have become united before final cross-segmentation. The chromosomes are arranged in the post-synaptic nucleus end to end, but the pieces which form the parts of any one bivalent are not necessarily adjacent to each other until after the first transverse segmentation. This view harmonizes somewhat incompletely with that of Farmer and Moore, Mottier, Schaffner, Juel, and others.

The spirem of *Pinus* and *Thuja*, as indicated above, becomes a regular homogeneous band in which only the slightest evidence of the longitudinal split remains. It is wound evenly throughout the cavity and consequently thrown into a number of loops (Figs. 10, 11). That the number of loops corresponds to the number of bivalent chromosomes cannot be stated definitely, although there may be some such relation. The spirem thickens very considerably and signs of cross-segmentation become quite evident. In some nuclei the number of loops is now almost identical with the reduced number of chromosomes (Fig. 14). This figure is drawn from *Thuja*. The nucleus is shown almost entire and the loops are quite as regular as any yet observed for this or any other genus. It is also quite evident here that the sides of the loops are double in certain places, although usually the double

nature is concealed. From a study of the chromosomes just after cross-segmentation of the spirem and before the segments have thickened to their final form it becomes evident that two pieces have become in some way approximated to form the bivalents. That these two members are the sides of the loops there seems little doubt. There are, however, many nuclei in which such a looping of the spirem with such diagrammatical regularity cannot be observed. In such nuclei the looping is far less regular. The spirem often has not become entirely regular, but has retained to a considerable extent its lumpy nature and the longitudinal split has not entirely disappeared (Fig. 13). The spirem consists of short loops, but there are often stretches of considerable length in which there seems to be no tendency to form loops. These pieces often reach almost across the nuclear cavity (Figs. 13, 16). That the chromatin content is being drawn out of the parts of the cord and deposited in other is often quite evident (Fig. 15). This often presents the appearance of dark, deeply staining parts connected by thin strands, the whole appearing like a reticulum (Fig. 15). It seems that the spirem breaks up and pieces are brought together and fuse. There is no such massing of the spirem in this condition to a central aggregation as is often apparent in other nuclei. It seems quite likely that the spirem completes its cross-segmentation without further change and gives rise to the bivalent chromosomes. This method, while varying somewhat from the condition in which regular loops are formed, is seen to admit of the same interpretation, namely, that two pieces previously arranged 'tandem' in the spirem become approximated to form a chromosome.

The chromosomes formed as shown in Figs. 17, 18, 19 now enter upon the period of shortening and thickening. Some of the pieces are variously oriented toward each other, and it becomes evident that many of the forms observed at this time consist of the two sides of a loop which have become bent together and applied closely, while others are completely broken apart or the line of demarcation is quite distinct. The pieces are often twisted over each other at this time, but, as shortening and thickening takes place, they seem to straighten somewhat and appear more like straight pieces lying alongside each other, or as links or rings. The Y and X forms and the figure eight are also to be found.

There is nothing in the spirem of either *Pinus* or *Thuja* to indicate the relation which the somatic chromosomes bear to each other. Miss King ('07) has shown that in *Bufo* certain chromosomes have the typical ring-form which has been commonly observed in all studies of the heterotypical chromosomes, while others are of a dumb-bell shape. In that form the spirem from which the chromosomes are formed is never found to be split longitudinally, and thus all possibility of their origin as demanded by the popular theory of conjugation side by side is precluded. Miss King ('07, Figs. 28–31) shows very clearly that the chromosomes are arranged 'tandem'

in the spirem and that they may either give rise to rings by looping on themselves (Fig. 31) or dumb-bells by cross-segmentation without looping (Fig. 28); the cardinal points of this being, that no conjugation of spirems takes place before synapsis, that the chromosomes are united end to end to form the bivalents, and no longitudinal split is observed. A similar method of conjugation of chromosomes has been given by Strasburger ('04) for *Galtonia* and by Mottier ('07) for *Tradescantia*.

The chromosomes are now located mainly near the nuclear membrane which has up to this time retained its characteristic appearance. The chromosomes are connected more or less by thin delicate strands which often reach out to the nuclear membrane or even to the cytoplasm. The nuclear membrane now begins to fade out and spindle fibres crowd into the cavity. At this time the segments have shortened almost to their final form, although there is yet considerable difference in their relative sizes. The segments in *Thuja* become relatively much shorter and thicker than in *Pinus*, and accordingly the stages following spindle formation are much more difficult to follow than in *Pinus*. The orientation of the chromosomes into the equatorial plate now takes place. This occurs quite rapidly and the chromosomes become arranged almost in a single plane. This is quite strikingly true of *Thuja*. The structure and relation of segments is not so apparent at this time, but a careful examination reveals all of the forms observed in the prophase. The chromosomes are closely crowded together and some are in a plane slightly above or below the rest. There is no indication that the segments have begun longitudinal fission as yet (Fig. 20). The stage of the spindle plate lasts some time in *Thuja*, as the division goes on comparatively slowly in this form. The spore-mother-cells undergo this division about the first week in February. Entire sporangia have been observed in which every cell exhibited the chromosomes in the plate of the spindle. Such cones brought into the laboratory and placed in a warm place finish the division in a few hours.

The manner of attachment of the spindle fibres, as well as the behaviour of the chromosomes during the metaphase and anaphase, was found to be in general harmony with the well-known accounts of Strasburger ('00) and Mottier ('03). Miss Ferguson ('04) states that the spindle fibres are never attached at the free ends of the segments, but invariably at some point along the fused part, but this does not appear to me to be the case. The chromosomes are usually arranged tangential to the spindle and sheaves of fibres are attached to the segments either at the free ends or at the point of adherence of the two segments. A few of the chromosomes stand radially to the spindle.

The separation of the daughter segments now takes place. Two well-known theories are still held as to the manner in which the separation is effected. Miss Ferguson ('04) maintains that the sister segments are not

separated in this mitosis, but that the original longitudinal fission again becomes evident and the daughter segments separate along this line of fission, thus one half of each somatic passes to each daughter nucleus and this mitosis effects an equational division. After a careful study of such stages, as shown in Figs. 21, 22, 23, the opposite view has been adopted, namely, that the segments separate in such a way that one of the halves of the bivalent passes entire to each daughter nucleus, thus bringing about a reducing division. Fig. 21 shows the beginning of metakanesis; the segments shown are not the longitudinal halves of the daughter segments, for, as will be shown in later stages, longitudinal fission has not yet taken place. The chromosome in the middle was a ring-shaped one, while those to the right and left were composed of two straight pieces which may have been oriented in any of the ways mentioned above. The ends are drawn together as they converge toward the poles and in many cases they are so close together that they may appear fused. This is, however, more apparent than real. The segments at this time are quite thick, but appear slightly more drawn out than when in the spindle plate before metakanesis begins.

After the segments entirely separate from each other and move toward the poles, each is seen to split longitudinally. The appearance of this fission is often very much delayed, but it takes place quite generally, as shown in Fig. 22. It may be readily seen here that the segments begin their fission at the equatorial end and separate along the entire length. The equatorial ends diverge somewhat and the characteristic V, U, or double V is formed. The second chromosome from the left represents a ring-shaped form in which longitudinal fission has taken place, giving rise to the double U, while that lying next to it may be due to a long rod-shaped one which has bent on itself at the place to which the spindle fibres were attached. Sometimes it happens that one of the granddaughter segments slips past its mate, the chromosome at the left, but they more than often remain together. Frequently their polar ends seem to be fused, but this does not appear to be the rule. In Fig. 23, the granddaughter chromosomes are shown just as they arrive at the poles. Longitudinal fission is now complete. The segments have not yet contracted and show the same long-drawn-out appearance which is typical of this stage of mitosis in *Pinus*. It is evident here that longitudinal fission has been complete, although the ends of some of the segments are very closely applied to each other and often seem fused. The granddaughter segments now approach very close to each other, and it becomes difficult to trace the individual members. A more or less complete spirem is formed, and about the same time the nuclear membrane is laid down. This spirem which has been formed by end-to-end fusion of the segments soon gives signs of dissolution, and, with the growth of the daughter nucleus to its full size, the spirem has entirely lost its identity and a com-

plete resting nucleus is formed (Fig. 24). The chromatin now exists in the state of rather large irregular lumps connected by linin threads, the whole presenting an appearance not strikingly different from the resting nucleus of the spore-mother-cell of the first mitosis (Fig. 25).

The question quite naturally arises at this point as to the possibility of the complete re-formation of the granddaughter segments during the homotypic mitosis; also as to the meaning of the longitudinal fission of the prophase and the relation which it bears, if any, to the fission which occurs during the anaphase. It has been quite generally assumed that the fission which occurs in the chromosomes as they pass to the poles is but the reappearance of that which takes place during the prophase, but it will be remembered that this former fission had apparently entirely disappeared at the time of the final shortening and thickening of the chromosomes, so that only a theoretical basis exists for such a supposition. Those investigators who believe in the morphological continuity of chromosomes are not willing to admit that a complete resting stage is reached between the two mitoses, but nothing can be clearer than that such is the case in *Pinus*, as was pointed out by Miss Ferguson and confirmed in this work for both *Pinus* and *Thuja*.

The Homotype Mitosis.

The nucleus remains in this condition but a short time when there are once more signs of the formation of a spirem. Many of the delicate anastomosing strands are drawn in and the chromatin becomes evenly distributed along the connecting threads, in which case a rather broad spirem is formed. This spirem is never entirely complete, as there are always many folds and occasional cross branches (Fig. 26). It is always, however, a single cord. Miss Ferguson states that this spirem during its final segmentation into chromosomes forms in loops across the spindle fibres, and that as cross-segmentation takes place the loops undergo longitudinal fission, and that accordingly two U-shaped segments are formed from each loop. These daughter segments then separate completely and form the chromosomes of the homotype mitosis. I have examined many nuclei in all stages of this mitosis in both *Pinus* and *Thuja* with this one point constantly in mind, but have never been able to find any evidence at all convincing that such a condition occurs in either genus. The spirem is formed as a single cord and so it always remains. The chromosomes are formed by cross-segmentation of the spirem (Fig. 27). The shape of the chromosomes vary considerably. They are generally, however, rather long rods, either straight or hooked at the end, or bent in the middle. The compound curve is occasionally met (Figs. 27, 28).

Before cross-segmentation has taken place the nuclear membrane has disappeared, and spindle fibres crowd into the cavity (Fig. 26). The spirem then segments transversely into the chromosomes. These chromosomes

do not seem to occur so regularly in pairs as described for many other plants, but are scattered throughout the cavity. Orientation into the spindle plate soon follows (Fig. 28). The segments are generally placed tangential to the spindle and do not all occur in the same plane (Fig. 29). It is often difficult to determine the two segments which make up the respective chromosome. When the chromosomes finally become arranged in the plate of the spindle they are very much crowded together, and it is very difficult to follow all of the segments. It is quite certain, however, that from their manner of segmentation and orientation no such longitudinal fission occurs as that described by Miss Ferguson. The chromosomes often reach across the equatorial plate, so that it becomes difficult to determine toward which pole some of the segments will eventually pass. The fibres are attached to the ends in the great majority of cases, but in a few instances they may be attached near the middle. In a number of cases observed a few of the segments were seen to stand radially to the long axis of the spindle. The segments are entirely free from each other and each now constitutes a chromosome. At the beginning of metakinesis a much-tangled appearance is often presented and some of the segments seem to be standing radially to the axis of the spindle. This is due to the fact that some of the segments which pass to a given pole lie on the opposite side of the nuclear plate and are bent somewhat by the pull which seems to be exerted by the fibres. As the segments separate and pass to the poles, all of the forms observed in the prophase are met with. The segments seem to straighten as they move apart. This is always true of the forms which are attached to the ends. As the segments pass toward the poles the equatorial ends of neighbouring chromosomes diverge, while the polar ends come close together, thus forming the characteristic V. This figure is therefore due to two different chromosomes, which are so closely applied to each other as to appear fused. Some of the long rods which were attached to the spindle near the middle form the U shape, but the straight rods forming V's are much the commoner form (Figs. 32, 33). After the segments reach the pole they shorten and become closely applied to each other and their identity is soon lost.

There is nothing, apart from purely theoretical reasons, in either of these genera, to indicate that the segments which occur in the homotype mitosis are the same as the ones which were present during the anaphase of the first. As already stated above, the chromosomes of the first mitosis lose their identity completely before the spirem of the second is formed. The loss of identity is as complete as is that which occurs in the resting nucleus preceding the heterotype division. What basis exists then for asserting that the homotype chromosomes are identical with the granddaughter segments of the first mitosis? This centres again on the question of the individuality of the chromosomes. If the chromosomes are not capable of a complete

re-formation as maintained by Farmer ('07), Mottier ('03), Foot and Strobell ('07) and others, then it becomes at once evident that the segments are formed anew and that the material primordia, pangens, or chromosomes are again shuffled and grouped into new combinations. Such a view leaves meaningless the longitudinal fission of the spirem during the prophase of the first mitosis, as well as that of the chromosomes during the anaphase. These two fissions have generally been regarded as one and the same, and as being concerned with an equational division which is completed during the homotype mitosis. But this supposition demands the morphological continuity of chromosomes, an hypothesis concerning which the gravest doubts have been raised by the most competent investigators. The homotype division is not therefore, according to this view, analogous to a somatic division, the equational part of which took place during the preceding mitosis, but is in fact what it appears to be—also a qualitative division. The first division is qualitative and reductional.

General Considerations.

A more uniform interpretation prevails among botanists to-day concerning the phenomena of fertilization and reduction than at any time in the history of these most perplexing of all questions. That fecundation effects an approximate doubling of the number of chromosomes, half of which are furnished by each of the fusing gamete-nuclei, has long been known. Equally clear has been the knowledge that this number must be reduced at some time previous to the formation of the next succeeding generation of germ-cells. The method by which this reduction takes place has been a very difficult problem, and, although no solution has yet been offered which satisfies all of the phenomena observed by different investigators, there is a growing tendency toward a common view of the essential nature of the process as a whole.

At present the view which was formerly held by many of the best investigators, that the reduction is accomplished in the resting nucleus, and after two longitudinal fissions of the spirem the chromosomes are distributed essentially in the same way as in somatic divisions, has been almost, if not quite abandoned. This view not only failed to harmonize with the observed facts of the behaviour of the chromatin, but also failed to furnish a suitable basis for theoretical considerations based on the known facts of heredity. It has, accordingly, been gradually superseded by a growing belief, which is now almost universal, that a true reducing division takes place as proposed by Weismann several years ago, although it is quite generally agreed that the reduction occurs in the first rather than the second mitosis. This change of conception was brought about largely by a more careful study of the early prophase of the first mitosis, as well as by a more general accept-

ance of the theory of the individuality of the chromosomes; for, if the chromosomes are to be regarded as individuals, it is clear that reduction can take place only when one of the two tetrad or maturation divisions is transverse.

The belief in a true reducing division is not new, but its general adoption as a true process of meiotic division is comparatively recent. Probably the greatest stimulus to research along this line was exerted by the re-discovery of the Mendelian principle of the segregation of characters in certain inbred hybrid forms.

As long ago as 1884, Heuser asserted that there occurs in *Tradescantia virginica* a transverse division of the chromosomes in the first mitosis, and that this mitosis effects, therefore, a qualitative division. Korschelt ('95) reported similar conditions in the annelid *Ophryotrocha puerilis*, but neither of these investigators succeeded in convincing subsequent observers of the accuracy of his work, and it was passed unheeded. It was not until experimental investigators had re-established the Mendelian principle of character segregation in hybrids that botanists began with renewed energy the search for a reducing and a qualitative mitosis.

Too much credit cannot be given to the plant breeders for the valuable contributions they have made in the form of statistical reports of the outward manifestations of hereditary characters in many species of plants. This work, while furnishing valuable criteria for the cytologists, has done more to dispel the idea that fecundation consists in the chemical union of the two fusing gamete-nuclei than could ever have been accomplished by the student of cytology alone. The students of experimental research on heredity have been driven to assume the material primordia of characters which the cytologists have been able to demonstrate as existing in the germ nuclei, and thus the two working from directly opposite sources have reached the same conclusion, a coincidence which must be regarded as of the greatest significance. There seems no longer any doubt that there exist in each gamete-nucleus certain material primordia which are in some way responsible in the determination of the characters of the resulting organism. That these primordia, or determinants, are definite chemical compounds seems quite probable, although nothing explicit can be offered along this line at the present time. All the experimental work of the past argues strongly against the idea that these primordia fuse at fecundation in such a way as to give rise to an entirely new hereditary substance. On the contrary investigation seems to bear out the view that the primordia are merely mixed in a mechanical way, but without chemical union. Whether these material primordia of characters be regarded as the chromosomes themselves, as has been generally the case, or as the smaller particles of which they are composed, as suggested by Weismann and variously modified by De Vries, Strasburger, Mottier, Farmer, and others, it seems quite

generally agreed that they are separate, distinct entities which persist in their individuality from cell generation to generation.

Although Rabl ('85) was the first to attribute to the chromosomes the property of individuality, Boveri ('08) first formulated the hypothesis which has had such far-reaching influence on all subsequent work in cytology. While Boveri's hypothesis was not accepted without reserve by all investigators, it has been quite generally adhered to, and it is only just recently that it has again been brought seriously into question. Recent research has caused a growing belief that too much speculation has been based on the theory of the individuality of the chromosomes.

All work of the past which has been done in support of the hypothesis has endeavoured to show that the chromosomes are capable of a complete re-formation after having first entirely lost their identity in the resting nucleus. The researches of Herla ('93), Haecker ('95), Moenkhaus ('04), Ruckert ('04), Zoga ('95) and others furnish us with abundant evidence that the maternal and paternal chromatin remain distinct from each other for at least several divisions in the developing embryo. Ruckert has also shown that the chromatin of the germinal vesicle appears in two distinct groups, and he suggests as possible that these groups may represent the maternal and paternal elements which have remained distinct throughout the entire life of the organism down to the formation of the egg. It has been shown for *Pinus* by Miss Ferguson ('04), Blackman ('98), Chamberlain ('04), and others that the gamete-nuclei do not unite at fecundation and that the chromosomes of the first cleavage division of the egg appear in two groups. Blackman ('04) and others have shown that the two nuclei in the *Uridineae* do not fuse for many generations after their association in the same cell, in fact not until the close of the life cycle—the meiotic phase. Moenkhaus ('04) showed that the chromosomes actually remain distinct in a certain hybrid fish until the third division of the embryo. Probably the strongest argument in favour of the persistence of the chromosomes from generation to generation as distinct morphological identities is to be found in the report of Rosenberg ('04) for *Drosera* hybrids. This writer has shown that in the hybrid sporophyte produced by crossing *Drosera rotundifolia* with *D. longifolia*, which have respectively ten and twenty chromosomes, the chromosome number is thirty as would be expected. At the time of the next succeeding reduction division, however, the number is not fifteen bivalents as would be found ordinarily after normal fecundation, but ten bivalents and ten univalents. Since the chromosomes of the two original species differ in both form and size, it is possible to recognize the chromosomes of the maternal and paternal ancestry. It is found, therefore, that the ten bivalents are formed of one from each ancestry respectively, and that the ten univalents are the ten from the one ancestry which did not find mates in the other group. This evidence seems to point conclusively

to the fact that the chromosomes have retained throughout the life of the organism down to spore-formation their individuality, and have simply become associated in pairs during meiosis.

Less certain evidence is that derived by Farmer ('07) for hybrid ferns. In *Polypodium Schneideri*, the result of a cross between *P. aureus* and *P. elegantissumus*, the chromosome number at meiosis is not a mean between that of the two species from which it was derived, but is considerably larger. There are in this case also several unpaired members. The large number of chromosomes of this hybrid, as well as the original species from which it was derived, renders the results less convincing than that of the *Drosera* hybrid.

Another piece of evidence which has had great influence on this favourite hypothesis is the presence in certain insects of differential chromosomes which are so different from all others that they may be followed readily from one division to another. It is, in fact, to this phenomenon more than to any other that we are largely indebted for our present view of synapsis and reduction, first suggested by Montgomery ('01) and later developed by Sutton and Boveri.

Wilson ('05) concludes from a study of these differentials that the chromosomes are 'definite, well characterized entities which show the most marked individual characteristics of behaviour, which in some manner persist from one cell generation to another without loss of their specific character, and which unite in synapsis and are distributed in the next ensuing maturation division in a perfectly definite manner'. This writer regards, therefore, the question of the individuality of the bodies in question as definitely settled. He considers the problems of cytology to be 'problems of the comparative morphology and physiology of the chromosomes with the ultimate aim of attempting their specific correlation with the phenomena of heredity and development'. Wilson himself, working on this hypothesis, has developed his well-known theory of sex determination. But in the plant world, although differences have often been noted in respect to both size and shape of chromosomes, no such differentials occur as are found in the cases cited above. Cardiff ('06) has suggested that it is highly probable that one chromosome in *Acer* is structurally different from the others, but he does not offer any speculation as to its significance. Likewise Gates ('07) ventures the assertion that a differential occurs in *Oenothera*. Full confidence cannot be placed in this work, however, until it is more certainly reported and definitely confirmed for many genera of plants, a condition which it seems safe to predict will never be realized. In almost any spore-mother-cell the chromosomes differ in form and size, but that this difference is constant enough to warrant any definite conclusions concerning their correlation with heredity and development does not seem probable.

Briefly then the theory of the individuality of the chromosomes finds its strongest support in the behaviour of the chromosomes in hybrid forms, coupled with the presence of the peculiar differentials and the observed methods of separation and distribution during the two divisions attending reduction. Conclusive as the evidence in favour of the hypothesis seems to be, the recent contributions of Foot and Strobell ('07), Farmer ('07), and of Mottier ('07), have strongly questioned its validity. These writers have pointed out certain objections to the hypothesis, chief among which may be mentioned the difference in the number of chromosomes typical of a species, and the number of characters into which the species may be analysed which behave as distinct allelomorphs. This objection was also recognized by Weismann, but has been given new meaning through the definite proof afforded by recent experimental work. It has been urged that each chromosome may be regarded as a material primordium for a group of correlated characters, and this is to a certain extent borne out by experimental evidence. For example, Mendel ('65) found that in *Pisum* a grey, grey-brown, or leather-brown colour of the seed-coat always occurs in connexion with violet-red blossoms and reddish spots in the leaf-axils. This correlation of all the characters in groups becomes lost, however, when the entire number and relative independence of the allelomorphs is considered. It seems necessary to associate some smaller unit with the occurence of unit characters.

Brauer ('93) thought that the chromomere rather than the chromosome should be regarded as the element which retains its individuality from cell generation to generation. This theory has recently received new recognition from Mottier ('07) and later from Farmer ('07). Mottier has suggested that the smallest visible chromatin granule be taken as the unit which retains its individuality. These units he designates as pangens. The next higher aggregation of granules is the chromomere. Farmer ('07) regards the chromomere as the distinct entity and material primordium of the unit characters. He considers the chromosome as made up of a number of organized chromomeres, but the same chromomeres do not necessarily always go together to form a chromosome. There is, therefore, according to this view no persistence of the chromosomes from cell generation to generation as distinct individuals, but rather they are formed anew at each succeeding division out of the individual chromomeres which have retained their identity.

Much importance has always been attached to the fact that the chromosomes are constant in number, and much speculation has been based on the number of chromosomes in the germ-cells of certain species. Farmer ('07) has shown that in certain ferns chromosome numbers are present which deviate greatly from the number typical of the species from which they have sprung. He concludes that this number must have arisen

through a rearrangement of the substance of which the chromosomes are formed, and regards this as proof of the variable nature of these bodies. The same writer concludes that the 'number and form of the chromosomes typical of any species is evidence rather of the organizing function of the cell as a whole than of the independent nature of the chromosomes themselves'.

In connexion with this theory of the individuality of the chromomeres and variability of the chromosomes another set of phenomena must be taken into consideration, namely, the origin of the chromosomes from the male and female ancestry respectively, and the possibility of their recognition in the resting nucleus. As implied above it has been quite generally accepted that the two sets of chromosomes remain distinct throughout the growth period in the life of the organism and unite only at the time of meiosis. This supposition has been attacked by Mottier ('07) and also by Foot and Strobell ('07). Mottier ('07) sees no reason for regarding the chromatin as remaining in such a complete state of segregation, since there is but one known genus (*Pinus*) in which the maternal and paternal chromosomes do not entirely lose their identity at the time of fecundation. He also points out the fact that any interchange or pairing of pangens may as readily take place at this time as during meiosis. There is no time it seems in the entire process which is so well fitted for distribution and rearrangement as the stage during which the chromatin exists in the finely divided state connected by delicate anastomosing linin threads.

Foot and Strobell ('07) have shown for *Anasa tristis* that the morphological identity of the chromosomes is entirely lost during the rest period of the first spermatocyte, but that among the eleven chromosomes which emerge from this resting nucleus there are three which differ so markedly from the others that they are readily distinguished from the early prophase to the telophase of the second spindle. These forms are the so-called eccentric chromosome, the microchromosome, and a large cross-shaped one. These authors conclude, however, that this is not an indication of the morphological continuity of these forms, for it is argued that if during the growth period the chromosomes pass over into the form of a chromatin reticulum, skein, or granules, there is no ground for asserting that the chromosomes have retained intact their morphological continuity. It is also maintained that no basis exists in fact for asserting that the paired bivalents are two of the somatics of the maternal and paternal ancestry which have remained together. It is urged that the chromosomes which make up the heterotype bivalents are formed anew out of the material of the chromosomes of the preceding generation, a view which harmonizes with that set forth by Mottier ('07) and by Farmer ('07).

It is a fact worthy of note that Foot and Strobell have based their conclusions on the same data from which Montgomery, Stevens, Wilson,

and others have derived their well-known theories of synapsis and reduction which are so directly opposed. In *Anasa tristis* much importance has been attached to the fact that in certain of the microchromosomes there is a considerable variation in size from those demonstrated in any other of their preparations (Foot and Strobell, '07, Plate II, Photo 3). In this cell the other chromosomes are somewhat smaller than usual. It is pointed out that if these two parts of the bivalents be interpreted as having been derived respectively from the maternal and paternal ancestry, then 'it becomes necessary to accept the theory that not only two individuals can show exactly the same marked variations in the same chromosome, but that these two rare cases should unite, a condition which seems unlikely'. According to this argument it also becomes necessary that the eleven daughter bivalents of a group should be as constant as the equality in size of two daughter bivalents, and this is shown not to be the case in *Anasa*. These authors also point out the fact that if such differences in size be due to parental differences, then we should rarely find two daughter bivalents exactly alike, but that this equality of daughter bivalents is a conspicuous fact. Briefly, then, the evidence derived from a study of the differential chromosomes of this form is not in harmony with the well-known hypothesis as it has been variously developed by different investigators.

By attributing the property of individuality to the finer units of which the chromosomes are composed, we do not by any means preclude the possibility that some chromosomes may from cell generation to generation retain to a certain extent their morphological continuity. It cannot be doubted that in cells in which divisions are taking place rapidly, the chromosomes of the preceding generation can often be almost certainly recognized as irregular lumps which have not become so finely dissected as may happen when divisions are further removed. My opinion is, however, that too much importance has been assigned to this phenomenon by recent observers, for, if the chromosomes are seen to be without that persistent individuality which has been attributed to them, then, the so-called prochromosomes are seen to lose the significance with which they have been regarded.

Investigators have sought at different times to prove that it is possible under certain conditions to recognize the chromosomes of previous generations in the resting nucleus of somatic and germ-mother-cells. Rabl ('85) believed that he could recognize traces of the chromosomes in the resting nucleus, and he regarded the reticulum of such nuclei as having arisen by the giving off of anastomosing branches by the chromosomes, thus causing an apparent loss of identity. At the next division, however, the chromatic substance flows back through predetermined paths, and occupies the same position as before the loss of identity. He concludes, therefore, that the

chromosomes persist in the reticulum of the resting nucleus in the form of bodies with projecting processes.

The majority of investigators since the time of Rabl, while contending for the truth of the general hypothesis set forth, have failed to recognize anything comparable with the chromosomes in resting nuclei. Recently, however, Rosenberg ('04) and Overton ('05) have asserted that in certain genera it is possible to recognize in the resting stage of the nucleus a number of chromatic lumps which is equivalent to the number of somatic chromosomes typical of the species. For these lumps Overton ('04) has suggested the name prochromosomes. As already stated above it is not disputed that the chromosomes may for a time persist as rather large irregular lumps, but it seems quite probable that such appearances are due rather to growth conditions than that they represent a condition which has any great significance from the standpoint of heredity. For is it not true that, in at least the great majority of forms of both plant and animal so far thoroughly investigated, all vestiges of the structure of the chromosomes of the preceding generations are lost? The term prochromosomes, as applied to the chromatic lumps of the resting nucleus, is open to certain objections which are well founded. In all of the forms which Overton himself cites, it appears that the identity of these bodies is completely lost before final chromosome development takes place, so that it cannot be definitely stated that these bodies represent structures which are even approximately the same as the chromosomes of this division, as the name would imply.

In the case of *Podophyllum*, Overton ('05) says: 'Doch die Hauptsache ist, dass auch hier, gerade so wie bei den andern Pflanzen, Linin und Chromatinstruktur parallel zu liegen kommen'. And this, if it be true, relegates the entire speculation concerning prochromosomes to a position of minor importance.

This statement brings us also to a consideration regarding the origin of chromosomes from the spirem, concerning which there is a wider diversity of opinion than for any other stage of mitosis. It is maintained by one group of investigators, among whom Strasburger, Guignard, Berghs, Allen, Overton, and others are prominent, that the chromatin of the resting nucleus exists in a state of complete segregation, and that previous to synapsis, the maternal and paternal each arranges itself into a continuous spirem. These two spirems, lying close to each other, become arranged parallel throughout, approach very close together, and apparently fuse side by side. This fusion takes place during the synaptic phase. Following synapsis the spirems again separate to a greater or less degree, and segment transversely to form the bivalent chromosomes. Each part of a bivalent is therefore derived purely from the maternal or paternal ancestry. The other view which opposes this conception, and which has been supported by Farmer and Moore, Mottier, Schaffner, Juel, and others, denies the presence of two

spirems previous to synapsis, and asserts that the synaptic phase does not consist in the uniting of somatic chromosomes side by side. This view maintains that the chromomeres and the linin threads connecting them never become arranged in parallel rows, and that the chromatic content of the nucleus approaches the synaptic condition rather in the form of a reticulum of single chromomeres connected by single strands of linin. This chromatin reticulum becomes converted during synapsis into an endless spirem, which undergoes longitudinal fission shortly after it is formed, and then segments transversely into the chromosomes. The chromosomes consist of two pieces of the spirem which have either come together after cross-segmentation or were not disassociated, but remained together at the time of segmentation. These pieces become variously oriented toward each other, and so give rise to the heterotype forms typical of this mitosis, a condition which harmonizes perfectly with the observed facts in both *Pinus* and *Thuja*.

It will be seen, therefore, that these two views agree perfectly as to the nature of the bivalent chromosomes, but differ widely as to their manner of origin from the resting nucleus. It seems then that the questions of the origin and ultimate destiny of chromosomes cannot be regarded as finally and definitely settled. In this connexion one must not forget to take into account that, in dealing with a phenomenon of such wide occurrence as that of mitotic division, it is strongly probable that the process will be found to be uniform in regard to all of its essentials, although differing in minor details, else how shall we explain its significance?

The Idioplasmic Theory of Heredity.

In speaking of the chromomeres as the bearers of hereditary characters, it is, of course, implied that these bodies are responsible only by their influence on the less stable cytoplasm. No attempt will be made at this time to enter into a discussion of the various ways in which the idioplasmic theory, first proposed by Nagelei, has been developed. In some form or other it has held the centre of thought among students of heredity for the past thirty years. That the idioplasm, which is identified by all investigators with the chromatin, constitutes the entire mechanism of heredity has not been accepted by all investigators, and recent work is causing a growing belief that the cytoplasm of the egg at least is fundamentally concerned with the transmission of characters.

In its extreme form the idioplasmic theory assumes that every organism may be analysed into a certain definite number of unit characters, and that in the cells of the organism a number of material primordia may be found which correspond with such units. It is of course needless to say that such a condition has not been shown to exist, although it is known that many characters behave as distinct units. 'It is known that a definite kind of

protoplasm derived from the parents tends to run through a specific cycle of changes, during which it transforms itself into an individual like the one of which it once formed a part,' but whether these changes are dominated by any one part of this protoplasm more than any other is still questioned by a few investigators. All writers recognize the fact that the transmission of hereditary characters is, from the physiological point of view, but the recurrence in successive generations of like forms of metabolism. It is not clear, however, to just what these like forms of metabolism are due. It has been suggested by Fick that every species is characterized by a protoplasm which differs from that of every other. There is, according to this view, a species or individual protoplasm which is handed on unchanged from generation to generation. The differentiation always takes place in such a way that the resulting individual resembles the original of which it was once a part.

If it be true, as has generally been asserted by all students of biology, that the nucleus is the formative centre of the cell, that all processes of constructive metabolism are possible only through the nucleus, then it seems likewise evident that the same body may best be conceived of as responsible for the distinctive changes of metabolism which are manifested in the recurrence of definite characters, such as form, shape, and colour. So conceived, the idioplasmic theory is seen to rest on the assumption that the cytoplasm acting as the substratum of heredity is moulded and rendered specific by the idioplasmic characters of the nucleus. This relation which exists between cytoplasm and the nucleus must be regarded as taking place in accordance with definite chemical or molecular laws, always giving rise to the peculiar protoplasm characteristic of the species or of the individual.

This theory does not deny the fact that the egg-cytoplasm fixes the type of development, or that the cytoplasm is a factor in the transmission of hereditary characters, but maintains that the action of the cytoplasm, whatever it may be, has been determined by the idioplasm. If such a view be accepted, the question arises as to whether the units of the idioplasm are characterized by different activities, or whether they merely act as part of the homogeneous substance. As already implied above, the weight of evidence seems to show that there are permanent elements of the idioplasm which are distinct in structure and function. Whether these material determinants differ in their chemical or molecular composition is not known, but it seems that on this basis only can we conceive their true relation to be the transmission of hereditary characters. The students of cytology working hand in hand with the plant and animal breeders have established many of the fundamentals of heredity. It remains to be seen how much clearer the biological chemist will be able to render the conception.

SUMMARY.

1. The chromatin of the resting nucleus exists in the form of rather large granular lumps connected by delicate anastomosing strands of linin. The number of such lumps is always greatly in excess of the number of somatic chromosomes typical of the species. There is therefore no evidence to be found here in favour of the idea of prochromosomes.

2. The nuclear content approaches the synaptic condition while still in the form of a reticulum. No spirem is formed previous to synapsis.

3. The term synapsis should be used to denote the definite stage of contraction which is observed to occur always in spore-mother-cells and which is universally recognized as a normal phase in the process of meiosis.

4. Synapsis does not affect the conjugation in pairs of the somatic chromosomes of the maternal and paternal ancestry respectively arranged side by side.

5. As the chromatin thread recovers from synapsis, it is frequently seen to reveal a double nature and this is interpreted as due to longitudinal fission. Since this double nature is never prominent and eventually almost entirely disappears, it is not considered of paramount importance in the final development of the chromosomes.

6. The spirem becomes somewhat regular and forms loops in some cases, although the number of loops is not always the same as the number of bivalent chromosomes. Some of the pieces become approximated after cross-segmentation. The spirem often presents an extremely ragged reticulate structure just before cross-segmentation.

7. The bivalent chromosomes separate during metakanesis, one member passes entire to each daughter nucleus and thus affects a qualitative division. The retreating chromosomes are seen to split longitudinally as they approach the poles. It may be questioned whether this split bears a relation to the split of the early prophase, since that fission had seemed to entirely disappear.

8. The chromosomes of the first mitosis completely lose their identity in the daughter nuclei, which pass into a complete resting stage and thus all speculations as to their continuity in the following homotypic division is purely theoretical, having no basis whatever in fact.

9. The spirem of the second division is formed singly and never undergoes longitudinal fission as suggested by Miss Ferguson for the genus *Pinus*. The chromosomes formed from this spirem are rods, either straight, hooked at the ends, or bent in the middle.

10. The chromomere and not the chromosome is regarded as the idioplasmic unit which, due to its action on the unstable cytoplasm, is responsible for the appearance of characters.

11. The second division is probably qualitative, since the chromosomes are not conceived of as being capable of complete rehabilitation and it seems reasonable that the material entities become rearranged during the rest period.

I am indebted to Prof. D. M. Mottier for kind criticism and helpful advice during the progress of this work.

BLOOMINGTON, INDIANA,
March 25, 1908.

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EXPLANATION OF FIGURES IN PLATES XXIX AND XXX

Illustrating Mr. Lewis's Paper on the Chromosomes in *Pinus* and *Thuja*.

All figures were drawn from sections with the aid of a camera lucida and with a Lietz apochromatic 2 mm. objective, 1·30 apert. Figs. 8, 13, 14, 16, 18 with compens. oc. 18; all others with compens. oc. 12.

Fig. 1. Pollen mother-cell of *Pinus Strobus* prior to synapsis. The chromatin is disposed throughout the cavity in the form of rather large lumps. No indication of pairing of the chromomeres or the linin threads. The number of chromomeres exceeds the number of somatic chromosomes of the species.

Fig. 2. A cell from the same anther loculus. The nuclear network is beginning the contraction which indicates that the synaptic phase is approaching. No indications of a spirem are evident. The chromomeres show no disposition to unite in pairs. Two nucleoli are present.

Fig. 3. The nuclear content is becoming more densely contracted. The chromomeres are beginning to appear as if their material was being spun out along the linin strands connecting them. The content is still a reticulum.

Fig. 4. A cell from the same loculus as above. Many of the linin strands have been drawn in. The chromomeres have become somewhat evenly distributed along the linin strands and an incomplete spirem is present. Some of the chromomeres still show clearly at this time. No indication of fusion or conjugation of chromomeres or threads. The cells are still in tissue connexion.

Fig. 5. *Pinus austriaca.* Complete synapsis. The cell has rounded up and the nuclear content is in the tightly contracted condition. Nothing intelligible can be seen at this time.

Fig. 6. From the same loculus as Fig. 5. The synaptic mass is beginning to loosen. Loops project out from the dense mass into the cavity. The chromomeres have assumed a striking uniformity in size and form. The thread is clearly double and the chromomeres occur quite uniformly in pairs.

Fig. 7. Tangential view, same stage as Fig. 6. The double nature of the thread is evident but the parts have not separated from each other.

Fig. 8. Pollen mother-cell of *Thuja.* A slightly later stage than Figs. 6, 7. The spirem has become quite evenly distributed throughout the cavity. Its double nature is quite evident, and in a few places the halves tend to separate slightly from each other.

Fig. 9. *Pinus austriaca.* A complete nucleus is shown. No free ends appear, and it is certain that a continuous spirem exists at this time. The daughter halves of the spirem have not separated.

Fig. 10. Same stage from *Thuja.*

Fig. 11. Slightly later stage. *Pinus austriaca.* The spirem has contracted somewhat, becoming slightly thicker and more regular. It is distributed quite evenly throughout the cavity, and no signs are evident of a second contraction. This is the stage of the evenly distributed 'hollow spirem' of some writers.

Fig. 12. *Pinus austriaca.* Tangential view of nucleus. The spirem shows quite clearly its double nature. The sister halves have separated from each other at certain points. This nucleus represents an extreme condition that is met in only occasional cells.

Fig. 13. A later stage from *Thuja.* The spirem occurs quite regularly in folds or loops. It is more lumpy at this time than is typical of *Pinus.*

Fig. 14. *Thuja.* The spirem has become arranged here in a very regular series of loops. Some of the loops are broken at the outer end. There is a dense mass at the inner ends of the loops. Such regular stages are not typical and are met only rarely.

Fig. 15. *Thuja.* The spirem has given way to a quite ragged reticulum. This stage is frequently met in both genera.

Fig. 16. *Thuja.* Cross-segmentation of the spirem. Some of the pieces are quite long and are variously oriented toward each other.

Fig. 17. *Pinus austriaca.* Segments shortening and thickening not yet complete.

Fig. 18. Same of *Thuja.* The nuclear membrane has disappeared, and the cytoplasm is becoming somewhat fibrillar. There is at this time no evidences remaining of the early longitudinal fission.

Fig. 19. Same for *Thuja.*

Fig. 20. Mature spindle.

Fig. 21. Metakanesis. The members of the bivalent chromosomes are still attached at the ends and the longitudinal fission is not yet apparent.

Fig. 22. Anaphase in *Pinus Strobus.* The retreating chromosomes are undergoing longitudinal fission as they pass to the poles. Straight rods, V's, double U's, and rods bent at the end result.

Fig. 23. *Pinus Strobus.* The granddaughter segments have arrived at the poles. Some of the pieces are quite long drawn out. They are not closely oppressed at this time.

Fig. 24. Daughter nuclei. The chromosomes have almost completely lost their identity.

Fig. 25. Complete resting nucleus formed at the close of the first division. *Pinus Strobus.*

Fig. 26. Spirem of the second division. The spirem is much branched and irregular. It shows no signs of a double nature. The nuclear membrane has already disappeared and spindle fibres have crowded into the cavity.

Fig. 27. *Pinus Laricio.* The spirem has undergone cross-segmentation into the univalent chromosomes. The segments are rods either straight, hooked at the end, or bent in the middle. The spindle is not formed completely before cross-segmentation takes place, and the spirem does not undergo longitudinal fission.

Fig. 28. Orientation of the chromosomes into the spindle plate.

Fig. 29. The chromosomes are being brought into the plate of the spindle.

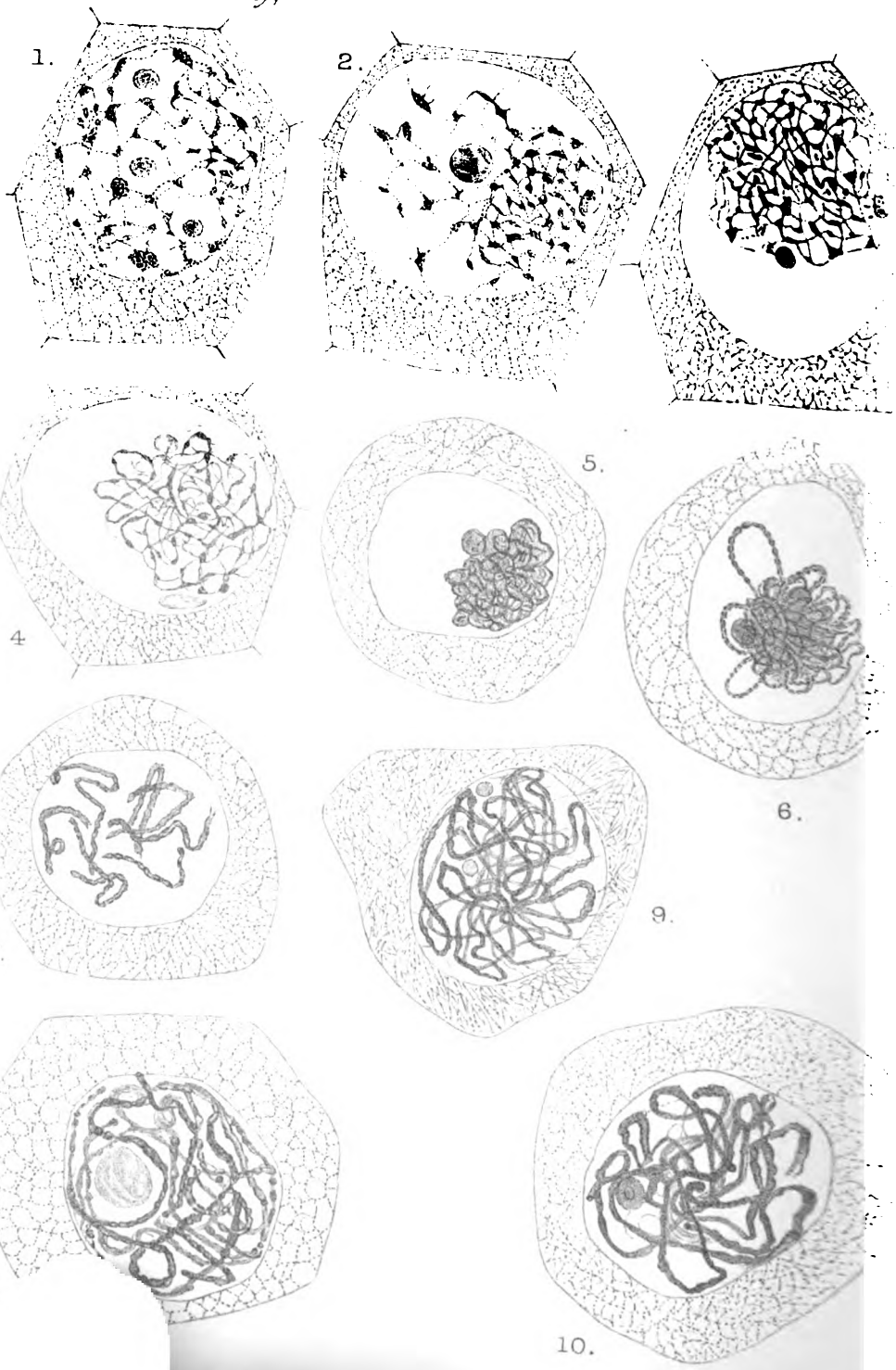
Fig. 30. The complete spindle plate. The chromosomes are much tangled, and do not all lie in the same plane.

Fig. 31. Metakanesis. The various shaped chromosomes may be seen at this time.

Fig. 32. Anaphase. The chromosomes are seen to be either straight rods, hooks, or U's. The V is due to two straight rods which have more or less completely fused at the ends.

Fig. 33. Anaphase. The segments show the same as the preceding figures.

Figs. 27, 28, 29–33 are taken from *Pinus Laricio.*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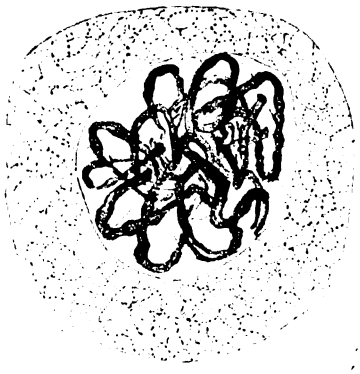

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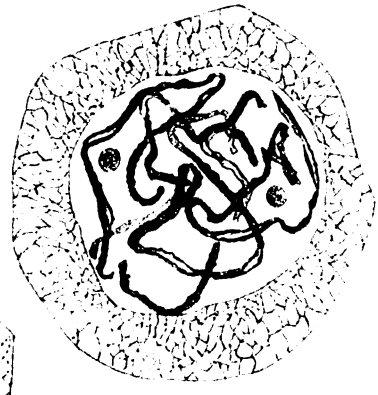

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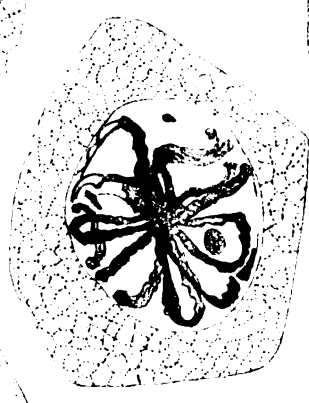

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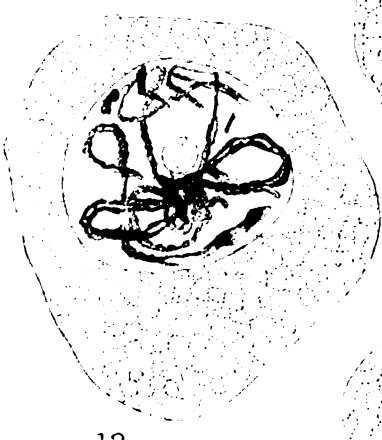

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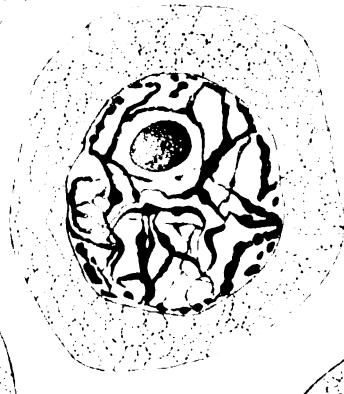

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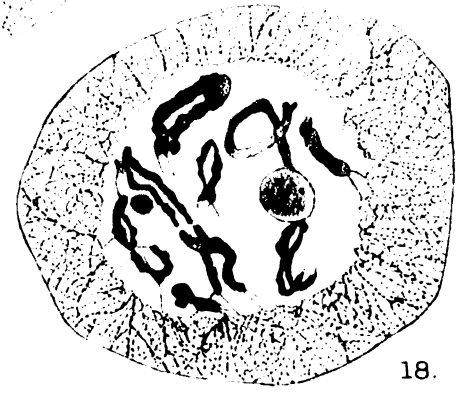

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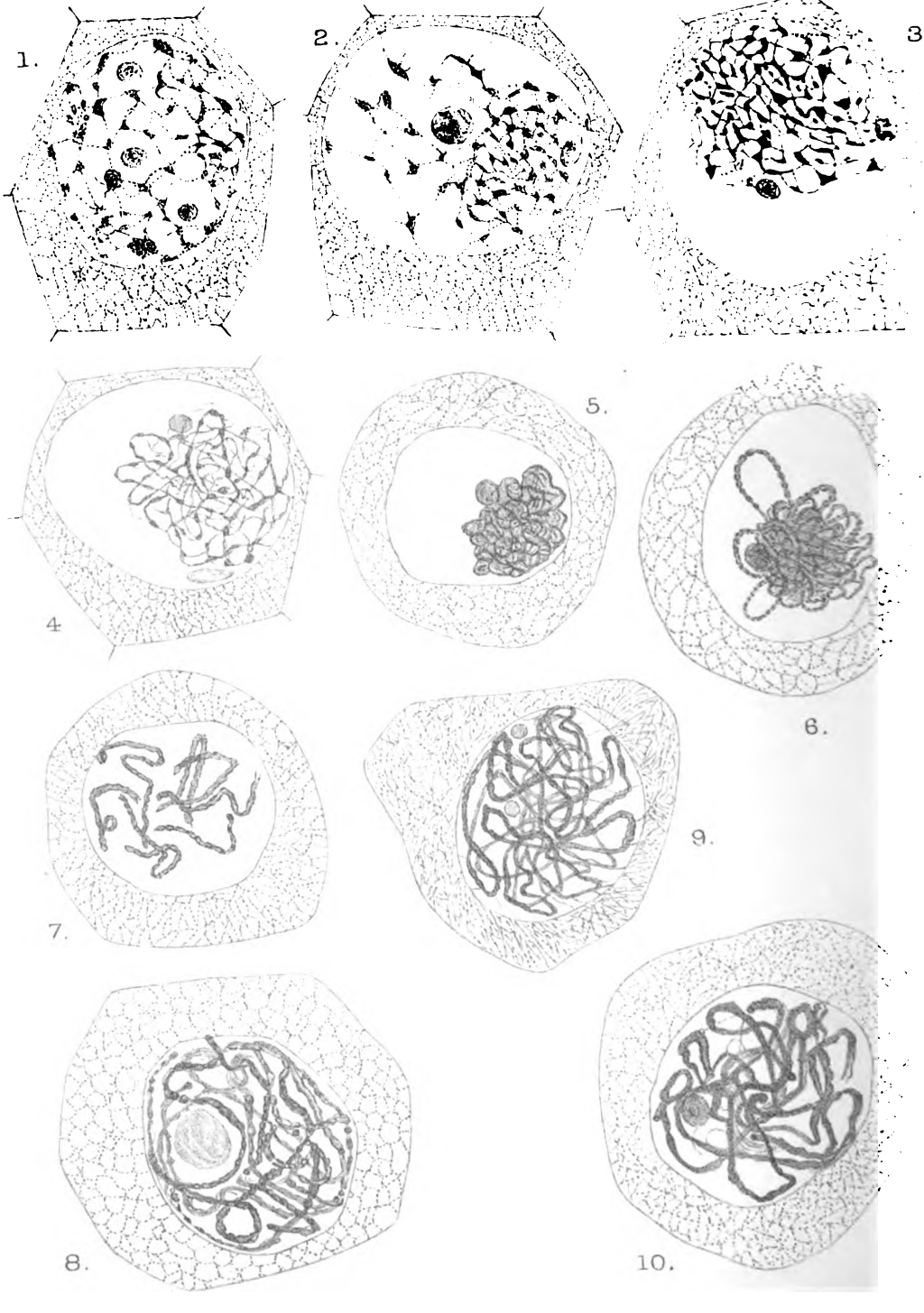
IN PINUS AND THUJA.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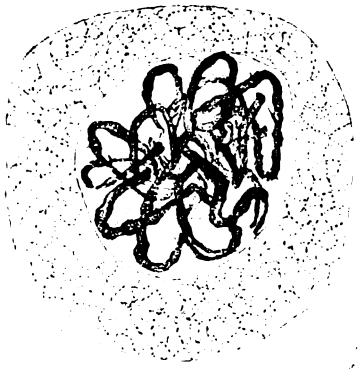

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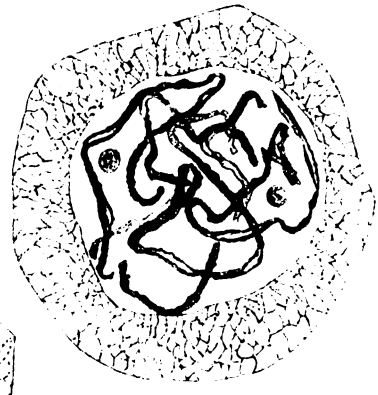

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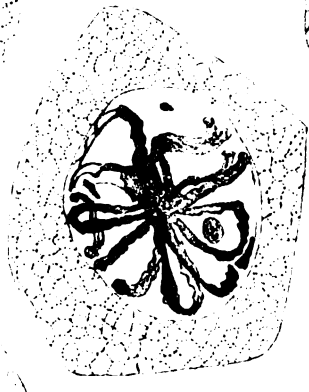

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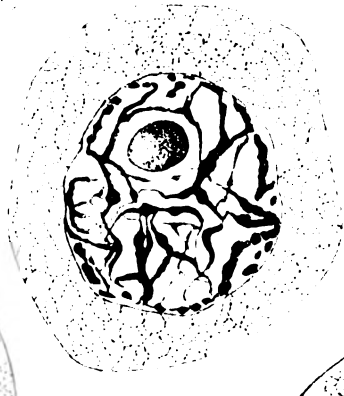

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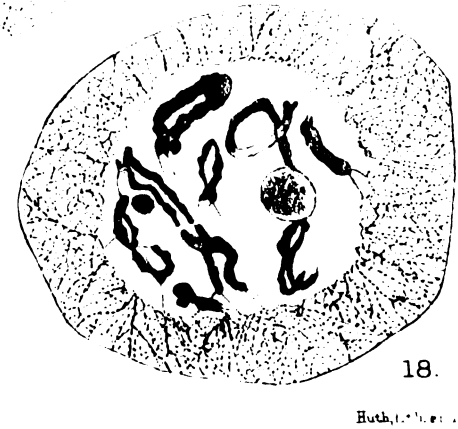

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IN PINUS AND THUJA.

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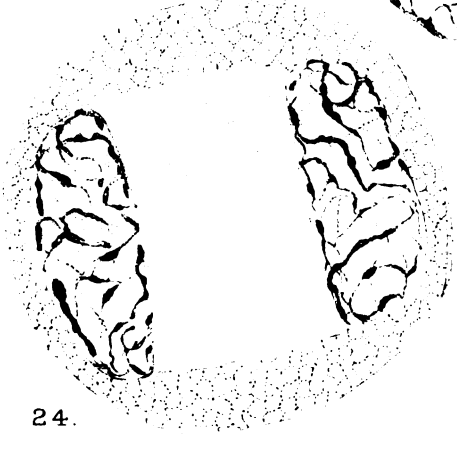

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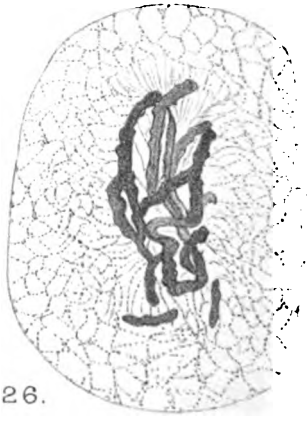

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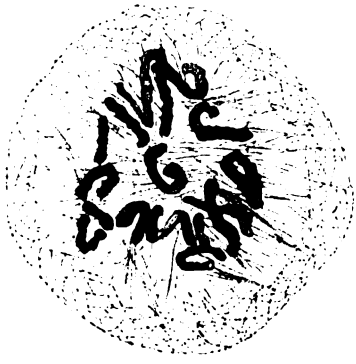

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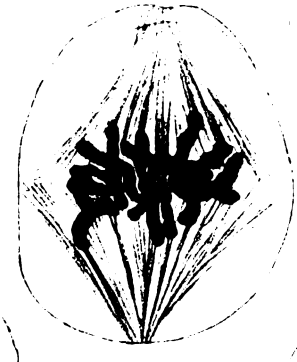

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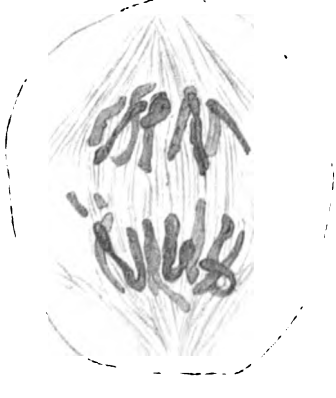

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NUS AND THUJA.

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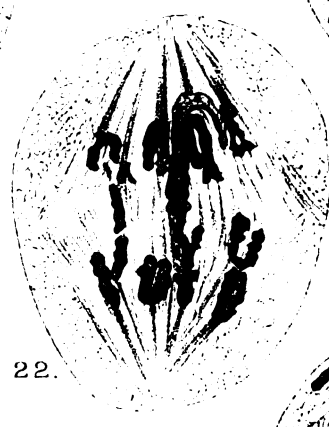

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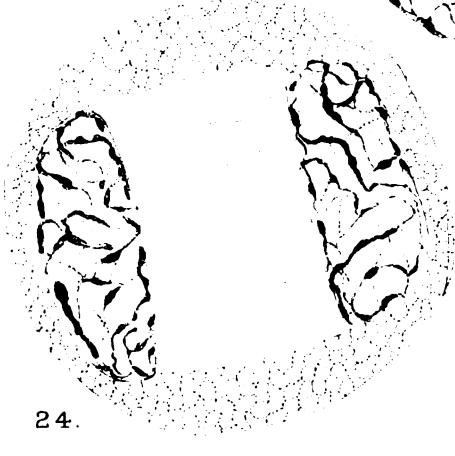

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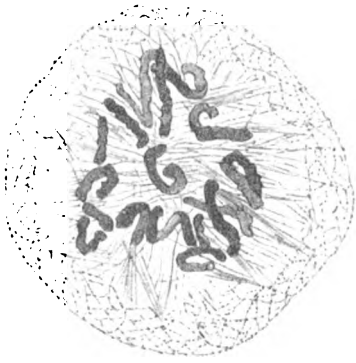

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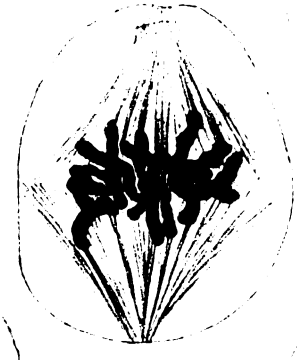

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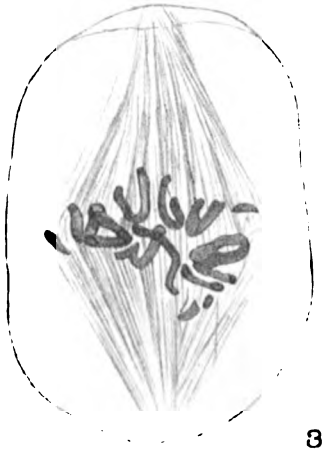

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NUS AND THUJA.

Temperature and Growth.

BY

W. LAWRENCE BALLS, M.A.,

Walsingham Medal, Cambridge, 1906.
Botanist to the Khedivial Agricultural Society, Cairo, Egypt.

With eleven Figures in the Text.

INTRODUCTION

THE object of the present paper is to analyse the effects produced upon the growth-process by the temperature factor.

The research was initiated by a casual observation [1], but developed into an attempt to determine the chemical effects of the temperature-factor by studying the pathological portion of the growth-temperature curve; and hence to demonstrate that the time-factor is chemical in its nature, and that its effects can be simulated immediately.

Isolated cells are preferable to bulky plant-bodies for such a purpose if the manipulative difficulties can be overcome, and I have employed as the subject an organism which has been under my observation for the past three years as a pest of the cotton crop—the so-called Sore-shin fungus [2]. It possesses one great advantage as a physiological subject, viz. the absence of any spore-formations, sexual or asexual, so that all the hyphae are both morphologically and physiologically equivalent. The chief novelty in the methods employed is the rapid rate of temperature change. By using so small an organism, and by recording its temperature with fair accuracy, it becomes possible to work through a long range of temperature in a short space of time. Errors due to the time-factor are thus minimized.

The observations given here, and the interpretations based on them, do not profess to do more than attempt to follow the lines along which explanations may be sought of the 'qualitative phenomena' of growth. We are not concerned with the 'quantitative phenomena'. Those internal factors, probably resident in the nucleus, which determine the rate of growth

[1] Khed. Agric. Soc. Year Book, 1905, pp. 184, 190. See also p. 33 *infra*.

[2] Khed. Agric. Soc. Year Book, 1905. Prel. Note, ibid., 1906. Field Notes, Preventive Measures.

Annals of Botany, Vol. XXII. No. LXXXVIII. October, 1908.]

for a cell under standard conditions, belong to a different category from those environmental factors which—the power to grow being granted—determine the relative amounts of that growth under varying external conditions.

It is hardly necessary to point out that the experimental and analytical methods employed are based on Blackman's 'Theory of Limiting Factors'. In 'Optima and Limiting Factors[1]', he points out that 'the way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when the process is being pushed towards the upper limits of its activity'.

Preliminary Work

In my first publication[2] concerning the Sore-shin fungus it was stated that the growth of the fungus was soon inhibited at 34° C. on agar media, and that this inhibition corresponded to the results obtained by raising the temperature of an infected cotton seedling. The wide interval between the 'inhibition temperature' and the death-point led me to examine the matter further, in mass cultures and in hanging drops. The apparatus in common use for the latter purpose was proved to be untrustworthy, and all the results obtained with it have been omitted from this account, being superseded by the later work here given.

Solid Media. The results were at first erratic when the cultures in agar streak tubes were repeated; growth might continue freely over the surface of the jelly, or it might cease after an hour or two, as in the first experiments. The cause of this irregularity was found in the texture of the jelly, the former being the case on soft agar, and the latter on agar which had become dry and tough through storage in the dry air of the laboratory.

Growth on soft agar at 34° C. was not normal; it was marked by a tendency to form aerial hyphae, which grew rather more rapidly than those on the agar, so that the marginal extension of the mycelial disk was carried on by the return of these aerial hyphae to the surface, slightly in advance of those which already formed the edge.

These facts accorded with the expectations based on the hypothesis of poisonous excreta accumulating in the jelly and thus inhibiting the growth of the hyphae, entirely or partly. Renewal of growth at a low temperature would be due to the removal of the excreta, either by decomposition or by diffusion.

Liquid media should not exhibit this cessation of growth until several days had elapsed, owing to the free diffusion of the excreta away from the vicinity of the hyphae.

Liquid Media. Cultures stored at 20° C. develop mycelium with a smooth appearance, the hyphae branching occasionally and being almost

[1] Annals of Botany, April 1905.

[2] Khed. Agric. Soc. Year Book, 1905.

straight[1]. When the surface of the liquid has been covered with mycelium, the hyphae grow up the sides of the flask and may even fill the interior with 'flying hyphae'. Resting cells are formed in abundance.

Cultures stored at 34° C. developed mycelium which had a fluffy appearance owing to the development of numerous short aerial hyphae, which were extensively branched and feathery. The long flying hyphae are not developed, resting cells are but sparsely formed, and growth ceases much sooner than in the cool cultures. This cessation of growth is commonly known to mycologists as 'staleness'.

In one experiment where the volume of the medium was about 100 cc. growth continued steadily for four days at 33° C., but on the fifth day the edge of the disk had ceased to grow. When these stale flasks were diluted with sterile water and re-inoculated the fungus grew freely again, although undiluted controls remained as incapable of supporting growth as before.

The time required for the completion of 'staling' is less at high temperatures than at low ones, and it is also affected by the volume of the medium.

Mode of growth. The growth of this fungus is seen to be entirely apical, and never intercalary, when examined under the microscope. A slight contraction may be noted in cooling a culture, but this is probably due to a change in the permeability of the protoplasm.

Hyphae taken from a cool culture show vacuoles, but these disappear on heating, and when a hypha is growing at high temperatures it appears to be homogeneous. The increment in length measured under the microscope thus represents fairly the actual increment in bulk of the protoplasm. Possibly the absence of vacuoles may facilitate the staling process.

The apical zone of growth is very small; the hyphae are not more than 10 microns in diameter, and the longitudinal extension of the zone is not more than 5 microns. Growth can be measured with considerable accuracy by taking some mark, bend, or side branch near the apex as a control point

The hyphae branch fairly freely and all branches of a hypha—which implies all the hyphae in a culture—show the same temperature relationship, as can be seen when readings are obtained simultaneously from two or more adjacent hyphae.

Chemical Composition of a Stale Culture. The substances which cause staling have not yet been chemically identified, but it has been proved that a culture does not become stale of necessity because its food supply has been exhausted.

For this purpose the Urea Medium was employed[2]; when all growth had ceased the flasks were re-inoculated for extra assurance of their inability to support growth, and the medium was then analysed quali-

[1] Khed. Agric. Soc. Year Book, 1905.

[2] Page 563.

tatively for its various known components. Every one of the original components was present, from Urea to Calcium.

This analysis does not dispose of an objection that some substance might be present in minute traces, too small to be identified by ordinary analysis, and that staling was due to its exhaustion. The results of dilution experiments with distilled water dispose of this criticism.

Apparatus.

Key to Diagrams of Apparatus.

Copper plate (*c.u.*) in black. Control-couple in its paraffin bed shaded

B. Control-bath.
C.c. Control-couple.
C. Observer's chair.
G. Galvanometer.
G.L. ,, lamp.
G.S. ,, scale.
H. Heating bath.
K. Circuit key.
L.1. Bunsen of *H.*
L.2. Heating lamp of *c.u*
M. Microscope.
M.L. ,, illumination.
O. Air space of chamber.
P. Stirring pump.
W. Waste water.
a. Asbestos sheet.
c.m. Culture medium.
c.u. Copper plate.
g. Glass carrier-slide.
w. Water.

The moist chamber. The chamber in which the fungus is grown under the microscope is the most important part of the apparatus employed in this research. Several different forms were devised for the purpose, but only one of these satisfied requirements.

The essential point is that no change whatever shall take place in the concentration of the liquid of the hanging drop. The only way to avoid such change is to immerse the chamber entirely in some liquid; so long as the cover-slip is exposed to the air, even when covered by a jacket, these changes are likely to take place, on account of the uneven heating of various parts of the chamber, and on account of the temperature-lag induced by the relatively massive objective.

The chamber used consists of three parts, as shown in Figs. 1, 2, 3, 4. A crystallizing dish containing water stands on a glass plate which is held in the jaws of the mechanical stage. Inside this water-bath stands a small glass beaker which holds about 30 cc. of culture medium. The moist chamber itself stands inside this beaker: it is made from the top of a test-tube, over which has been cemented a ¾ in. circular cover-glass, thus forming a cylinder which is open at the bottom; this cylinder is weighed down in the beaker by a bent and sealed tube full of mercury, to which tube it is fastened by a rubber band; a bent glass capillary tube passes down between the outer wall of the cylinder and the inner wall of the beaker and up inside the former to within a millimetre of the cover-slip; this tube serves to remove the air from the chamber and to introduce the gas required for respiration.

Before a new chamber is used it is soaked in warm water for two days in order to remove any soluble bodies from the sealing-wax which cements the cover-slip and from the thin rubber band.

Application of heat. The preliminary experiments referred to above were made in a Pfeiffer Hot-Box. I found, when the thermo-electric method of temperature measurement was adopted, that the temperature of the

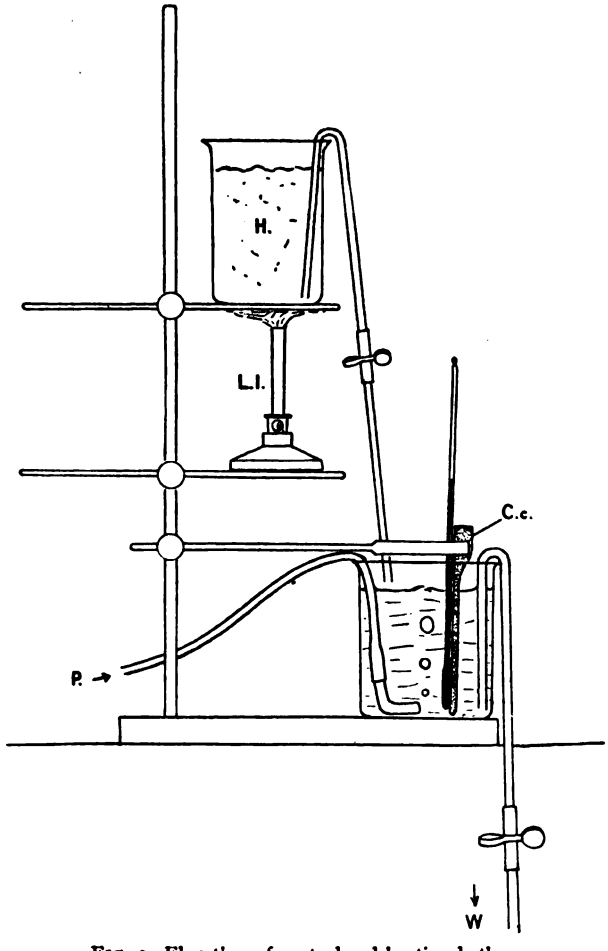
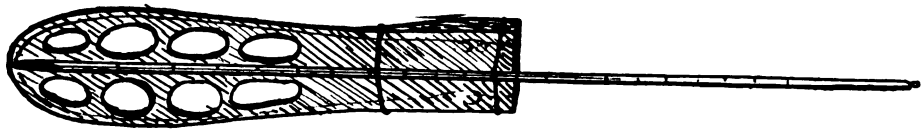


FIG. 1. Elevation of control and heating baths.

object on the cover-slip usually lagged behind that indicated by the thermometer in the air of the box by as much as 5° C. when the rate of heating was rapid. Since the temperature of the object could now be determined accurately without employing the cumbersome box, the latter was abandoned in favour of the following plan for applying the heat directly to the microscope stage.

The end of a long plate of thick copper lies under the end of the large glass slide which carries the water-bath. The outer end of this plate is supported on a stand. Injury to the microscope stage by the inner end is prevented by resting it on a sheet of asbestos, through which, as well as through the copper plate, a hole is bored for illumination of the object.



Control Couple in wax plate, with thermometer

Front elevation

FIG. 2 *a*.

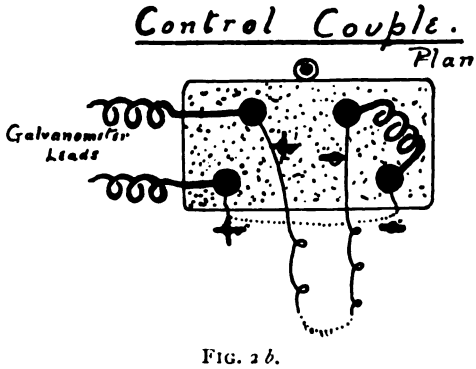
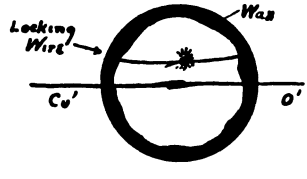


FIG. 2 *b*.



Cover-Glass.

FIG. 2 *d*.

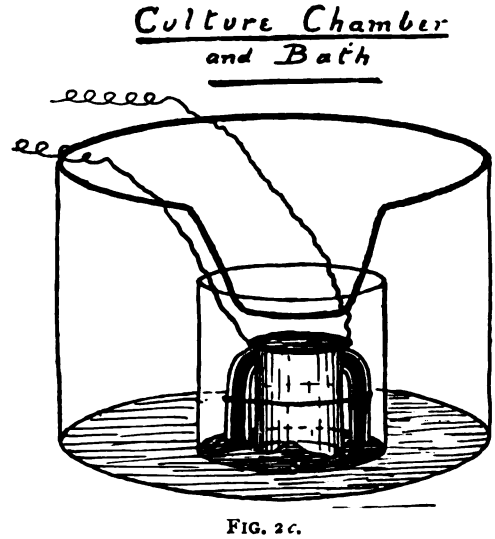


FIG. 2 *c*.

FIG. 2. *a*. Control-couple plate, with thermometer.
b. Plan of control-couple plate showing connexions.
c. Culture chamber.
d. Plan of cover-slip showing locking-wire.

Below the copper plate a small gas flame stands on the work-bench, and this flame can be moved to any point between the microscope and the outer support; the heat from this flame is conducted along the copper plate to the stage, and there heats the water in the bath, which in its turn heats the medium in the beaker, and the convection currents in this flow

over the cover-slip and slowly raise the temperature of the object. The rate of heating can be arranged for by adjusting the size and position of the flame. This method is very satisfactory in practice in spite of its crude appearance; the temperature of the cover-glass rises uniformly [1], and the rate of heating can be adjusted after a little practice; it is not suitable for constant temperature work, for which a water-bath should be employed

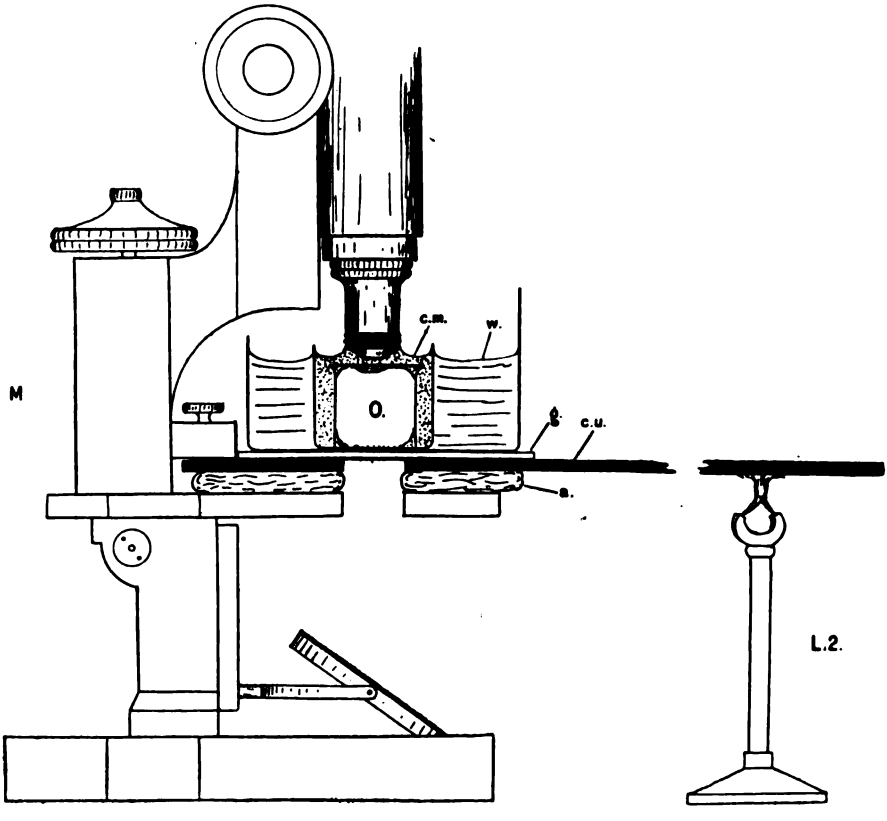


FIG. 3. Semi-sectional elevation of microscope and chamber.

Inspection of the records transcribed at the end of the paper will show its efficiency. Irregular gas pressure in the laboratory mains is the cause of the uneven heating shown in the first curve.

Temperature Measurement. Measurement of temperature is effected by Blackman's thermo-electric couples [2], using the method of balance.

One couple is varnished and embedded in a thin plate of 60°C. M. Pt. wax, and immersed in a water-bath. The ends of the couple pass into

[1] See curves given below.

[2] Blackman and Matthaei, Proc. Roy. Soc. B., 1905.

two of four mercury cups, which are cut in the thick upper end of the sheet. I have found this plan of embedding in paraffin to be much superior to insertion in a thin rubber tube on account of its greater durability; some of my couples have been in use for over six months, whereas the rubber-covered ones used to corrode through after a fortnight, and this durability is a great advantage in Cairo, for it takes six weeks to obtain couples

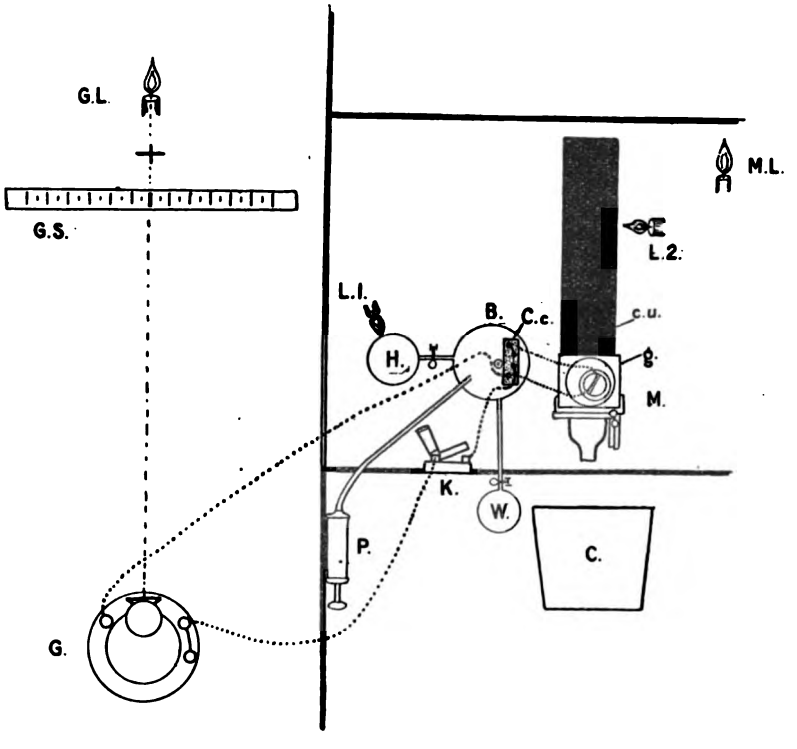


FIG. 4. General plan of apparatus, showing arrangement of various portions which has been found most convenient.

with which to repair the wreckage of a clumsy day. The wax plate takes rather longer to heat up to the bath temperature, but this could be avoided by reducing its size and strength.

The bath stands on the foot of a retort stand, resting on an asbestos cushion. On the bar of the stand are fixed clips for holding the paraffin sheet, a beaker, and a burner for heating the beaker. The beaker communicates with the bath by a clipped syphon, so that hot water may be added to the bath in order to raise its temperature when necessary. A

second syphon with a clip serves to carry off the waste water from the bath.

The other couple is also varnished thoroughly to avoid any galvanic effect, and is stretched across the moist chamber below the cover-slip, its ends passing out into the medium and air through the cement with which the cover-slip is fixed. A second plain, thin wire, which lies by the side of this couple, will be referred to later. The ends of the couple are rolled into springs and led through the air to the two unoccupied cups in the head of the paraffin sheet; to permit of this the microscope is placed close to the right-hand side of the bath. This grouping of the cups automatically prevents any error which might otherwise arise in the circuit from subsidiary thermoelectric effects, since the remaining connexions of the switch and galvanometer are paired.

Two of the like terminals of the couples are connected through the cups by a short piece of No. 20 B. W. G. insulated copper wire, while similar silk-covered wires lead to the galvanometer, with a Du Bois Raymond key in circuit.

The galvanometer is an Ayrton-Mather pattern D'Arsonval, made by the Cambridge Scientific Instrument Company, who are also the makers of the copper-constantin couples.

The galvanometer scale is graduated continuously (in millimetres) in order to avoid possible errors in the transcription of plus and minus signs; the zero is adjusted (at 30 cm. usually) by moving the scale, if necessary, before each experiment. The deflexion given for a difference in temperature of one degree Centigrade between the couples is about 60 millimetres, as the instrument was set up in this work, so that errors are not likely to be made in the measurement of temperature. In practice the readings are made to the nearest half-centimetre, because the accuracy with which the bath thermometer can be read is considerably less than this; an accuracy of 0·1° C. is all that is aimed at, while even this is less than the errors which are likely to arise in the measurement of slowly growing hyphae.

Culture Media. For general purposes the following formula has been used:—

Peptone	2%	Potassium hydrogen phosphate	0·05%
Liebig's Extract	0·5%	Magnesium sulphate	0·05%
Pure Cane Sugar	0·5%	Tricalcium phosphate	0·01%
		Distilled water	97%

When an exact knowledge of the chemical composition of the original medium was required, the Liebig and peptone were replaced by 1 per cent. of Urea. It may be pointed out for future study that the fungus seemed to find difficulty in growing on this medium at low temperatures (20° C.), but flourished at 32° C.

Solid media were made from these by the addition of 1·5 per cent. agar and 0·5 per cent. gelatine.

Experimental Methods.

Sterilization. After an experiment the chamber and beaker are washed and dried. Before the next experiment they are soaked in water at a temperature of 70° C. and rinsed with the culture medium. If these precautions are taken, bacteria rarely appear within the three hours which an experiment commonly occupies; the experiment is disregarded for critical purposes if any are seen.

Antiseptics cannot be employed on account of the difficulty of removing all traces of them from the wall and cement of the chamber.

Preparation of the Culture. The plan finally adopted was to inoculate a test-tube which contained 30 cc. of the medium under examination with a very small piece of mycelium from the aerial hyphae of an old, cool-grown, agar tube. The test-tube was left overnight at a temperature of 15° C. to 20° C. (unless some special temperature effect was under study) in order to allow new hyphae to develop. A disk would thus be formed of about 5 millimetres diameter.

A fragment of mycelium would then be taken from the margin of this mycelial disk, and placed in the chamber for observation. The size of this fragment was kept as small as possible, less than a millimetre in diameter and with a probable dry weight of about ·0001 grammes.

By thus allowing the fungus to develop in the medium to be tested, we ensure that the new hyphae shall contain the same percentage of the excreted bodies (if any) in their cell-sap as was contained in the medium.

Growth Measurement. The tiny fragment of fungus is clipped in position under the cover-glass by means of the thin wire which lies by the side of the chamber-couple. The chamber is then placed in the beaker and the latter is filled with the culture-medium from the test-tube; the air imprisoned in the chamber is sucked out if necessary, and oxygen or other gas is passed in by the capillary tube; the end of the latter is plugged with a glass rod.

The beaker is then placed in the water-bath, which is filled with water of the desired initial temperature and placed on the stage. The ends of the chamber-couple are led to their mercury cups.

The Zeiss D[1] objective dips into the excess of culture medium which, flowing over the surface of the cover-glass, protects it from the sudden changes of temperature formerly mentioned. The No. 4 Zeiss eye-piece contains a micrometer scale, one division of which is equal to ·003 mm. with this combination of lenses. This unit of ·003 mm. is the one employed in the curves transcribed at the end of this paper. Under favourable

circumstances it is possible to read to ·0003 mm. with this apparatus, but readings to less than ·001 mm. are usually to be regarded as doubtful[1].

After some time has elapsed the hyphae resume the growth which had been interrupted by their transference from the test-tube to the chamber; a suitable one is then selected for measurement. The requirements are—rapid growth in a horizontal direction, and the possession of some mark near the apex which may be used to check any general movement.

When the growth at a constant low temperature has been determined, the small flame is put under the copper plate, and the following readings are taken.

Readings. During the progress of an experiment the following readings are taken once every minute:—

(*a*) Time.

(*b*) Position of control-point of hypha on micrometer scale.

(*c*) Position of end of hypha on micrometer scale.

(*d*) Temperature of control-bath.

(*e*) Deflexion of galvanometer.

In practice the two latter are taken fifteen seconds earlier than the others, and a correction made, if necessary, when the curve is plotted.

When the rise in temperature of the chamber has carried the galvanometer to zero, the hot-water syphon is opened and the bath temperature raised about 1° C. To ensure thorough mixing of the newly-added hot water with that already in the bath, it is stirred up by blowing air through with a small hand-pump, the tube from this pump being weighted with lead to prevent it from jumping about in the bath and injuring the couple. The pump is worked with the left hand while the other is writing the note of the last reading.

One galvanometer reading is omitted after thus adding water, in order to make sure that the bath-couple, thermometer, and water are all at the same temperature. The amount added is adjusted by rule of thumb so that the galvanometer shall return to zero after two or three minutes.

The fungus may move slightly in the field; the adjustment to correct this is made by the mechanical stage. It is fortunate that the later stages of the experiment, when the fungus is near to the stopping-temperature, are rarely troubled by this movement, which seems to be due to the expansion of the copper plate.

The process might appear to be rather feverish from this outline, but in practice there is always time to spare. After some experience one experiment can be completed in about two or three hours, the last hour

[1] See curve 'Growth per Minute' in Curve 1.

being spent in taking consecutive readings, with practical certainty of a result. This does not, of course, include the time spent in the preparation of the culture.

Improvements. Though the apparatus has served its purpose up to the present, it suffers from a great many faults, chiefly, however, faults of convenience. By the help of an expert glass-blower, and by the use of steam-resisting cement, it should be possible to modify the chamber so that a number of cultures might be set up under aseptic conditions and examined successively, avoiding the loss of time which now follows transference from tube to chamber.

The heating apparatus also requires to be improved, as it is at present satisfactory for only rising or falling temperatures.

Experimental Results.

Key to Construction of Curves.

Typical sample experiments have been taken in each class.

Ordinates represent the actual length of the hypha, and not the growth per minute. The latter can be seen by inspection of column 5 of the experimental data attached to each curve. Growth per minute is given in addition on Curve 1 to indicate error of measurement.

When the control-point on the hypha has been changed during the experiment, the control-point has also been changed in plotting the curve, so as to give a continuous line representing the position of the tip of the hypha.

The curves had to be plotted fairly steeply in order to show clearly the falling off of the growth-rate. The temperature curve had to be on the same sheet, so that a glance along the ordinate would show the temperature at the time of growth-cessation. Consequently, in Curves 1 and 5, the growth-curve crosses the temperature curve, making an apparent confusion, which can be rectified by consulting the side indices.

The graphic ratio of the units of time, temperature, and length is the same in all the curve except No. 4, where the graphic temperature unit is doubled.

Curve 2 is half the scale of the rest, but with the same graphic ratio.

Curves 5*a* and 5*b* are plotted somewhat curiously in order to bring the graphic representation of growth-cessation in each to coincident points. This shows up the difference in growth-rate, time of appearance of the time-factor, and alteration in the stopping-point temperature more clearly than would have been the case with any other plotting.

Small crosses on the temperature curves represent times when the galvanometer was at, or very near to, zero, i. e. when bath and culture were at the same temperature, and the error of temperature determination at a minimum.

The Standard Cultures.

The temperature relationships exhibited by freshly grown hyphae when supplied with fresh culture media—the said hyphae not having been previously raised above 20° C.—has been made the subject of some thirty preliminary experiments. The results are quite consistent.

The growth-curves plotted from the results of these experiments show a steady acceleration of growth with rise of temperature, which corresponds fairly well to the expectation based on Van 't Hoff's law until 30° C. is reached. Above 30° C. this acceleration decreases, and the curve begins to flatten as the 'time factor' comes into noticeable operation. At a fairly definite temperature growth ceases and the curve becomes horizontal. The temperature at which this cessation of growth takes place is commonly called the 'maximum', but in view of the results obtained in this research it seems advisable to drop the word for the present; I shall refer to this point in the growth-temperature curve by the provisional title of 'stopping-point'.

The form of the upper part of the curve as it falls off to the horizontal is very variable; even at the same rate of heating it is not neccessarily the same, and it is flatter when the cultures are heated more slowly than the usual rapid rate employed. In a few cases the curve almost coincided with the theoretical expectation, the falling off of the growth-rate being scarcely noticeable until a minute before complete cessation. It will appear subsequently that these variations are probably due to differences in the freedom of osmosis from cell to medium, of the poisonous excreta.

The 'optimum' thus appears as the temperature at which the time factor and the temperature acceleration are in equilibrium, and it is not in any way a definite point. The word will not be used again in this connexion.

More interest centres round the stopping-point. When all allowances have been made for errors of observation, it is certain that with the present apparatus the point can be determined to within 0·3° C. of accuracy, or even less. The differences actually recorded are much more than this observational error; the stopping-points lie between 37° C. and 38·3° C., with one exception which will be described shortly. These variations will be seen at a later stage of the paper to be in all probability due to varying freedom of osmosis, provided that the rate of heating is approximately the same in each experiment.

The exceptional case, which in itself supports this idea, was the first reading of a double observation. The hypha almost stopped at 37·5° C., bent slightly at the tip, and continued to grow slowly until 40·5° C. was reached. The new portion bore the appearance of having burst out from the end of the hypha through a weak, though unbroken area in the cell-wall; this mark enabled the slight growth to be determined with absolute certainty. The second determination on this hypha after cooling down gave a reading of 37·4° C. The fact has some importance, for it shows that the cessation of growth is not due to any inability of the protoplasm to perform constructive metabolism beyond 38° C.

The principal point to notice, in view of subsequent results, is that in no case was the stopping-point depressed below 37·0° C.

STANDARD CULTURE. CURVE 1.

Standard culture: see Curve 1.

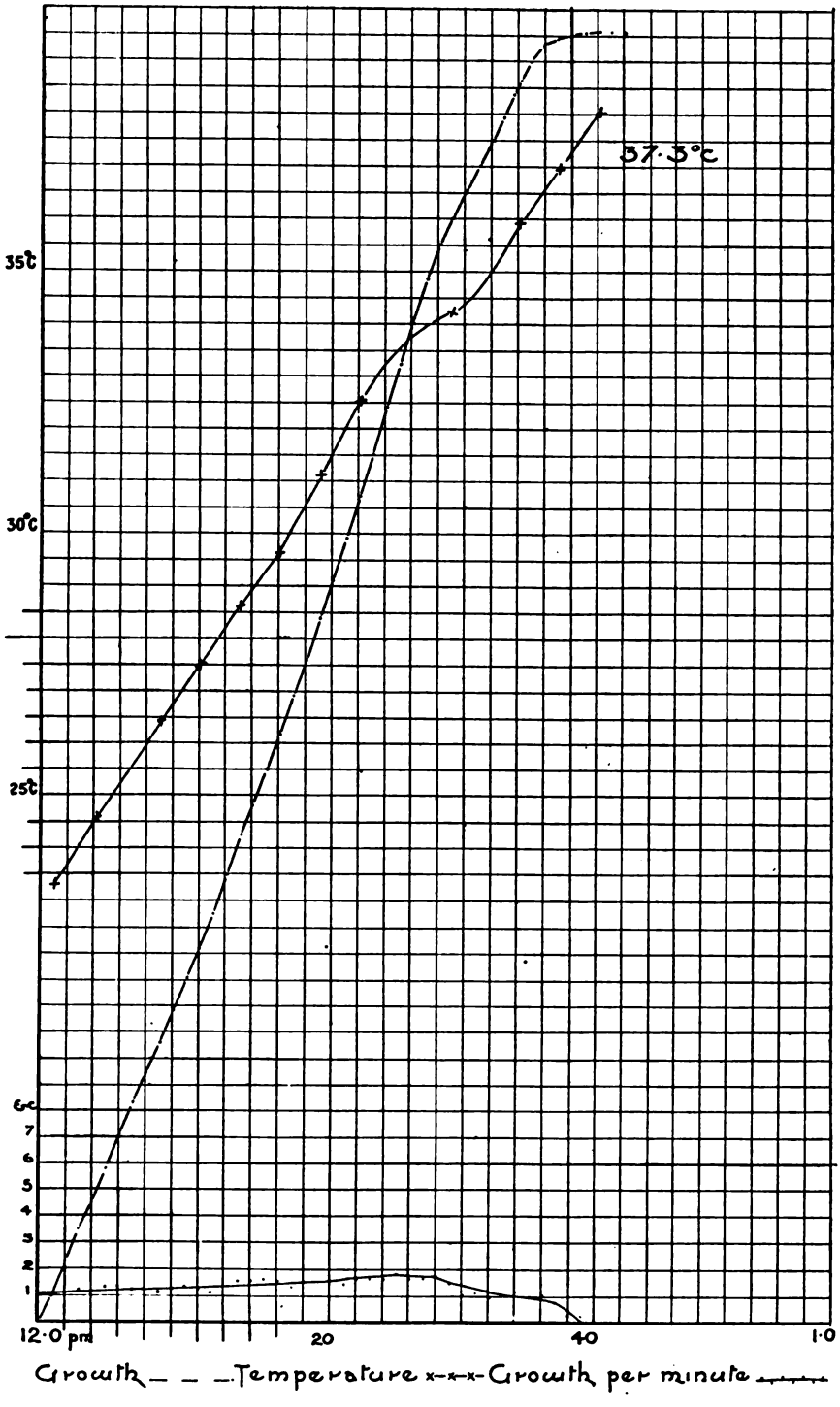
24/4/1907. 100 % fresh medium. Stored overnight at 20° C.

Set up 10 a.m. Pure oxygen. Galvanometer zero at 30 mm.

Lamp put in to begin heating at 11.57 a.m.

Readings of micrometer eye-piece scale in length-units of ·003 mm. (Columns 2, 3, 4, 5).

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
12.0 p.m.	3	26·9	23·9	—	—	—	
1	—	28	25	1·1	23·3° C.	29½	
2	2·7	29	26·3	1·3	—	—	
3	2·3	29·8	27·5	1·3	24·6	34	
4	2·7	31·2	28·5	1·0	—	31	
5	2·5	32·3	29·8	1·3	—	28	
6	2·2	33·3	31·1	1·3	—	—	
7	2	34·3	32·3	1·2	—	—	
8	2	35·6	33·4	1·1	26·5	33½	
9	—	36·8	34·6	1·2	—	31	
10	2·3	38·2	35·9	1·3	—	28	
11	—	39·4	37·1	1·2	—	—	
12	—	40·5	38·2	1·1	27·5	30½	
13	—	41·8	39·5	1·3	—	—	
14	2·2	43·2	41	1·5	28·5	32	
15	—	44·7	42·5	1·5	—	29	
16	—	45·8	43·6	1·1	—	—	
							New control.
17	11·0	31	20	—	29·4	32	
18	—	32·3	21·3	1·3	—	29½	
19	—	33·8	22·8	1·5	—	—	
20	—	35·1	24·1	1·3	31·1	31½	
21	—	36·8	25·8	1·7	—	29½	
22	—	38·2	27·2	1·4	—	—	
23	—	39·8	28·8	1·6	32·6	34½	
24	—	41·4	30·4	1·6	32·5	30½	
25	—	43	32	1·6	—	27	
26	—	44·8	33·8	1·8	—	—	
27	—	46·4	35·4	1·6	34·5	38	
28	—	48	37	1·6	34·4	36	
							New control. Opened lamp.
29	9·0	18·0	9·0	—	34·3	34	
30	—	19·3	10·3	1·3	34·2	31½	
31	—	20·3	11·3	1·0	34·2	29½	
32	—	21·4	12·4	1·1	—	—	
33	—	22·3	13·3	·9	—	—	
34	—	23·3	14·3	1·0	36·0	36	
35	—	24·4	15·4	1·1	—	33	
36	—	25·3	16·3	·9	35·9	30	
37	—	26·3	17·3	1·0	—	—	
38	—	27	18	·7	37·0	31½	
39	—	27·2	18·2	·2	36·9	29	
40	—	27·4	18·4	·2	—	—	
41	—	—	—	·0	38·1	31½	



CURVE I. Standard culture.

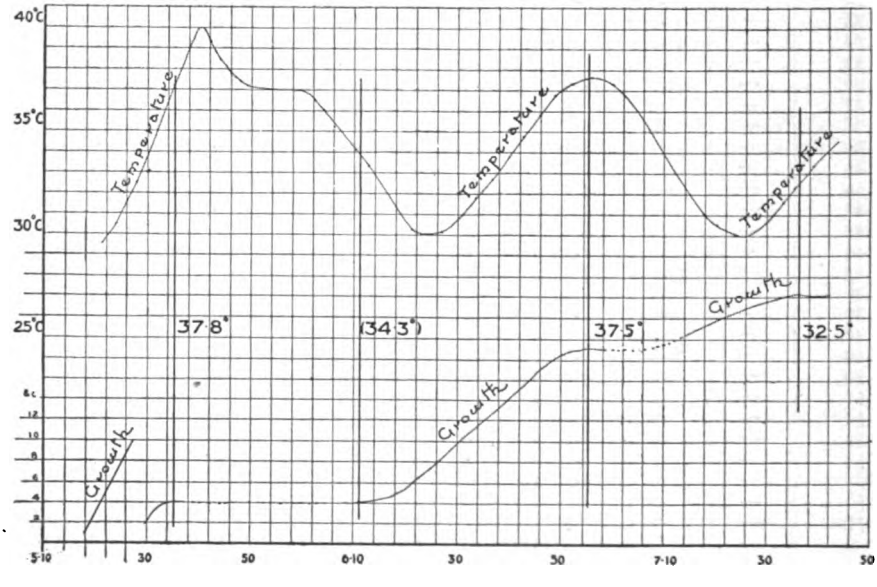
TRIPLE READING. CURVE 2.

Fresh Salomonson medium, 100 %, pure oxygen.
Tube inoculated evening of 9/3/07.
Set up 3 p.m., 10/3/07. Galvanometer zero, 30 cm.
Readings of micrometer eye-piece scale in length-units of ·003 mm. (Columns 2 3. 4, 5).

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
5.19 p.m.	—	32·0	30·0	1·1	29·4° C.	32	
20	—	—	—	—	—	—	
21	3	35	32·0	—	29 0	29	
22	2·0	34·8	·8	0·8	28·8	26	
23	0·0	33·6	33·6	0·8	—	—	
24	—	34·6	34·6	1·0	30·5	30·5	
25	—	—	—	—	·3	25	
26	2·0	38·8	36·8	1·1	—	—	
27	0·0	38	38	1·2	33·1	37·5	
28	—	—	—	—	32·7	31·5	
Changed to a branch of the same hypha.							
5.29	9·0	31·5	22·5	—	32·4	25·5	
30	10·0	33·0	23·0	0·5	—	—	
31	9·5	33·5	24·0	·5	35·7	38	
32	10	34·4	24·4	·4	·4	32·5	
33	—	35·0	25	·6	·1	26·5	
34	—	35·2	25·2	·2	37·1	30 !	
35	9·0	34·2	25·2	·0	36·9	29	
36	—	34·2	—	—	·5	23	
37	—	—	—	—	38·5	27 !	Partially re-
38	—	—	—	—	·1	24	[moved lamp
39	—	—	—	—	37·7	20·5	
40	—	—	—	—	—	—	
41	—	—	—	—	39·8	30	
42	—	—	—	—	·3	31	
43	—	—	—	—	38·8	—	
44	—	—	—	—	·5	—	
45	—	—	—	—	·1	30	
46	—	—	—	—	37·8	29·5	
47	—	—	—	—	·6	29	
48	—	—	—	—	·3	28·5	
49	—	—	—	—	37·0	28	
50	—	—	—	—	36·7	27	
51	—	—	—	—	—	—	
52	—	—	—	—	—	—	
53	—	—	—	—	—	—	
54	—	—	—	—	—	—	
55	10·0	35·3	—	—	37·5	27	
56	—	—	—	—	·1	27·5	Removed lamp.
57	—	—	—	—	36·8	27	
58	—	—	—	—	·5	26 5	
59	—	—	—	—	·2	26	
6. 0	—	—	—	—	35·9	25	
1	10·0	35·4	—	—	—	—	
2	—	—	—	—	36·4	28·5	
3	—	35·6	—	—	·1	39 !	
4	—	—	—	—	35·8	30	
5	—	—	—	—	·5	30·5	

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
6.6 p.m.	10·0	35·6	—	—	35·3	32	
7	—	—	—	—	·0	33	
8	—	—	—	—	—	—	
9	—	35·8	25·8	0·2	34·6	30	
10	—	—	—	—	34·2	31	
11	—	—	—	—	—	—	
12	—	—	—	—	33·9	37 ?	
13	10·0	36	26·0	0·2	·5	32·5	
14	—	—	—	—	—	—	
15	—	36·1	26·1	0·1	33·1	36	
16	—	·2	·2	·1	—	—	
17	—	·4	·4	·2	·4	32	
18	—	·7	·7	·3	·2	34	
19	—	37·0	27·0	·3	32·0	36	
20	—	·3	·3	·3	—	—	
21	—	·6	·6	·3	31·2	31·5	
22	—	38·0	38·0	·4	31·0	33·5	Lamp in.
23	—	·3	·3	·3	30·8	35	
24	—	·7	·7	·4	·6	36	
25	—	39·0	29·0	·3	·4	36	
26	—	·3	·3	·3	·3	33·5	
27	—	40·0	30·0	·7	·2	30·5	
28	—	·3	·3	·3	·1	28·5	
29	—	·7	·7	·4	30·0	25·5	
30	—	·9	·9	·2	—	—	
31	—	41·2	31·2	·3	31·1	31	
32	—	·6	·6	·4	·0	28·5	
33	—	·9	·9	·3	31·7	30	
34	—	42·2	32·2	·3	·6	27·5	
35	—	·7	·7	·4	—	—	
36	—	43·0	33·0	·3	33·3	34·5	
37	—	·3	·3	·3	32·7	31	
38	—	·8	·8	·5	·4	26	
39	—	44·2	34·2	·4	—	—	
40	—	·8	·8	·6	34·1	32	
41	—	45·2	35·2	·4	33·9	28	
42	—	·8	·8	·6	—	—	
43	—	46·1	36·1	·3	34·8	30	
44	—	·6	·6	·5	·5	26	
45	—	47·0	37·0	·4	—	—	
46	—	·4	·4	·4	36·1	32·5	
47	—	·8	·8	·4	35·9	29	
48	—	48·2	38·2	·4	—	—	
49	—	38·6	·6	·4	36·3	27·5	
50	—	39·0	39·0	·4	—	—	
51	—	·3	·3	·3	37·1	31	
52	—	·6	·6	·3	36·8	28·5	
53	—	·6	·6	—	—	—	
54	—	·7	·7	·1	38·2	35·5	
55	—	·8	·8	·1	37·9	33	
56	—	·8	·8	—	·6	30·5	
57	—	·8	·8	—	·3	28·5	Removed the
7. 0	—	—	—	—	36·8	24	[lamp.
7·14 About 1·5 growth, took fresh control-point on same hypha.							
14	13	22·6	9·6	—	—	—	
15	—	—	—	—	33·2	44	
16	13·0	22·9	9·9	—	·1	44·5	
17	—	23·3	10·3	·4	—	—	
18	—	·6	·6	·3	30·8	27·5	Lamp in.
19	—	·8	·8	·2	·6	28	

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
7.20 p.m.	—	24·0	11·0	·2	·4	29·5	
21	—	·2	·2	·2	·2	30·5	
22	13·0	·3	·3	·1	·1	32	
23	—	·5	·5	·2	·0	32	
24	—	·8	·8	·3	29·9	29·5	
25	—	·9	·9	·1	·7	28	
26	—	25·0	12·0	·1	·6	26·5	
27	—	·1	·1	·1	—	—	
28	—	—	—	—	31·4	37	
29	—	·6	12·5	—	·3	34·5	
30	—	·9	·9	·3	·1	32	
31	14·0	27·0	13·0	·1	30·9	29·5	
32	—	—	—	—	—	—	
33	14·0	27·1	·1	·1	·6	30	
34	—	·2	·2	·1	·4	26	
35	—	·3 ?	·3 ?	·1 ?	—	—	
36	—	·3	·3	·1	32·7	32	
37	—	—	—	—	·4	28·5	
38	—	—	—	—	—	—	
39	—	—	—	—	33·4	30	
40	—	—	—	—	—	—	
41	—	—	—	—	—	—	



CURVE 2. Triple standard curve.

The possible Limiting Factors in Standard Culture.

The essential points of the standard culture experiment are as follows:—

Minute fragment of mycelium develops hyphae in a relatively large volume of fresh, rich medium for twenty hours at a temperature which is not higher than 20° C.

A few of these hyphae are then removed, placed in the special damp chamber described above, and provided with a large excess of pure oxygen for their respiration.

The temperature of the chamber is not raised above 20° C. until growth has begun in the hypha under observation.

The temperature is then raised at least 1° C. in every four minutes.

All these precautions have proved to be considerably in excess of what is needed for any one of them.

In such a culture the possible limiting factors are somewhat as follows:—

1. Amount of available oxygen.
2. Rate of diffusion of oxygen through wet semi-permeable membranes.
3. Amount of carbon dioxide present.
4. Rate of diffusion of carbon dioxide.
5. Amount of available food material.
6. Rate of diffusion of food materials.
7. Rate of chemical change in the protoplasm, limited by temperature.
8. Water-supply.
9. Percentage of 'x' in the cell sap.
10. Rate of outward diffusion of 'x'.

This arrangement is not quite logical, because the amounts depend on the rates of diffusion, but it serves as a convenient mode of statement.

1. *The amount of oxygen.* The same results have been obtained with air in the chamber as when oxygen was employed, except in two early experiments when a very small air bubble was used for the respiration of a large mass of mycelium. In order to eliminate any possibility of this factor being limiting I afterwards used pure oxygen in the chamber for all experiments, though I believe it to be quite unnecessary.

2. *Rate of diffusion of oxygen.* This can hardly be a limiting factor at any time, for the use of pure oxygen has no effect on the stopping point, nor (as far as one can judge) on the rate of growth, though the partial pressure under these conditions is five times as great as in ordinary atmospheric air, and the rate of solution in wet membranes is correspondingly increased.

3. *Amount of carbon dioxide.* The culture solution is slightly alkaline, and remains so.

4. *Diffusion of carbon dioxide.* If the diffusion rate of oxygen into the cell is never a limiting factor, it is still less likely that the more soluble carbon dioxide should ever accumulate inside the cell in sufficient amount to influence the protoplasm, seeing that the air of the chamber contains no carbon dioxide whatever.

5. *Available food materials.* The culture solutions employed are fairly concentrated and can be diluted to at least a quarter of this concentration without affecting the stopping point, so that we may assume that there is ample food in the solution, and also (6) that this food passes into the cell by osmosis at a sufficiently rapid rate to satisfy all the cell's demands.

7. *Rate of chemical change.* There is some evidence in the curves obtained that the growth function obeys Van 't Hoff's law, within the limits imposed on it by the rapid staling which takes place at high temperatures. The cessation of growth is obviously not due to any destruction of the protoplasmic machinery, as is the case at the death-point; this is shown by the aberrant result of 40·5° C., and by the normal behaviour of hyphae which have been heated up as far as 41° C. and then cooled down again. Some effect seems to be produced on such hyphae, however, for the rate of growth is noticeably slower on the second heating, even though the stopping point be practically unaffected [1].

8. *Water supply.* Is never limiting.

8, 9. The hypothesis on which these results find their only present explanation is that some substance (or mixture of substances) is formed by the protoplasm in katabolism, and inhibits growth by its accumulation. Some of the temperature-growth phenomena of the organism depend on the amount of this substance which is present in the cell, which amount is again dependent on its rates of production and of removal.

The formation of this substance takes place at low temperatures as well as at high temperatures, but with far greater rapidity in the latter case.

The hypothesis approximates to a theory in that this katabolite has been isolated from the organism, and some of its properties determined; it has not yet been isolated chemically, nor identified.

Throughout the rest of this discussion this body will be referred to, for convenience, by the designation of 'x'.

Effects of previous exposure to high temperatures.

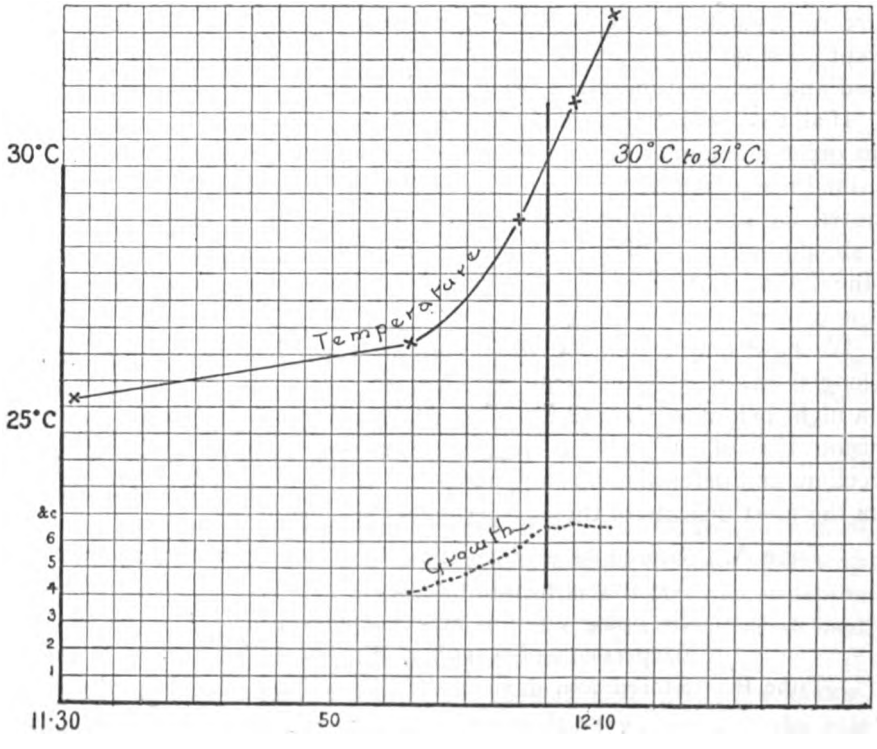
Having considered the temperature-growth phenomena under the most favourable conditions obtainable, we will turn to the effect of two modifications of these 'standard conditions' which throw considerable

[1] Curve 2.

light on our hypothesis. The first of these is the effect of prolonged exposure to 'sub-maximal' temperatures.

The effect of such exposure on the morphology of the fungus, and on the time required for the completion of staling, has been described previously. We now proceed to note the effect on the stopping-point.

A difficulty in the experimental method might be noted here in order to excuse the inaccurate temperatures employed; owing to the erratic gas pressure in the laboratory mains, and the absence of a pressure-



CURVE 3. Inoculated tube stored at 34–35½°C. for twenty-four hours. Curve represents the fastest hypha obtainable.

regulator, it is not possible to keep the Hearson incubator at a perfectly constant temperature. A nominal temperature of 33° C. is actually one of 32–34° C.

The curve plotted from a culture which has been stored at 33° C. before being transferred to the damp chamber for study is similar to that obtained in the standard cultures, except that the rate of growth is slower, and that the stopping-point is lowered.[1] On account of the irregularity

[1] Curve 3.

of the incubator it is not necessary to give the details of each experiment, but in every case some effect was produced, the stopping-point falling to

36·7° C., 34·0° C., 32·5° C., and 29·8° C.

in four experiments.

The offered explanation of this is that the body 'x' has accumulated rapidly in the cell, either in not sufficient amount to check growth, or else checking it completely. The presence of pre-formed 'x' in the cell has the effect of depressing the stopping-point, because it shortens the time required for the accumulation of the 'inhibitory percentage' of 'x'. The corollary of this phenomenon is recorded on p. 559, where the temperature is kept constant and the time prolonged, instead of the temperature being raised and the duration of the experiment reduced to a minimum.

If all that we have assumed about 'x' is true, we should be able to detect it in greater amounts in media which have contained mycelium at sub-maximal temperatures, than in media containing mycelium which had been exposed for an equal time to lower temperatures; in the former case more 'x' should be free to diffuse osmose out into the surrounding medium than in the latter. At present our only test for the presence of 'x' is the biological one.

We have only to remove the mycelium which has formed during the prolonged exposure, reinoculate with a fresh cluster of resting cells, store for a night below 20° C. until the cells of the new mycelium contain the same concentration of 'x' as the medium, and then remove a fragment of mycelium and determine its stopping-point in the usual way. The figures from the most delicate of these experiments are given:—

Tube A. Stored seven days at 20° C.
Volume of medium, 25 cc.
Stopping-point of new inoculation, 37·3° C.
Depression of stopping-point, negligible.

Tube B. Stored four days at 22° C.; one day at 33° C.; and two days at 28° C.
Volume of medium, 25 cc.
Stopping-point of new inoculation, 36·5° C.
Depression of stopping-point, at least 0·5° C.

These results show that 'x' diffuses out into the surrounding medium, and that it is formed more rapidly at sub-maximal than at low temperatures.

The length of time required for the completion of staling is greater at low temperatures than at high ones (p. 559), because less of 'x' is excreted in a unit of time, and it is greater in large amounts of culture medium than in small amounts, because more 'x' has to be formed in order to raise the concentration of 'x' in the medium to the inhibitory percentage. The

extreme case of the latter is seen in the cells of the cotton seedling, where the volume of the culture medium is so small that staling takes place in a few hours at so low a temperature as 30° C.[1]

Dilution of stale media.

If the idea of increasing concentration which has been sketched above be true, we ought to be able to simulate increasing staleness by adding the stale media to fresh media.

Before doing so we have to eliminate the possibility that the ratio of 'x' to the available food might have some subsidiary effect. This was done by four parallel experiments, using a solution which was on the verge of complete staleness:—

50 % Stale ;	50 % Fresh ;	stopping-point,	32·1° C.
50 % „	50 % Water ;	„ „	32·5° C.
50 % Fresh ;	50 % „	„ „	37·1° C.
100 % „	. . . . .	„ „	37·5° C.

Hence, if any such effect is exerted it is inconspicuous beside the effects of adding 'x'.

The first experiments gave erratic results, on account of the slow destruction of 'x' by boiling[2], and owing to the time wasted by this the results of the dilution experiments are not of a quantitative nature at present. The transfer of stale media from one tube to another is now effected through a flame, thus avoiding the necessity for subsequent sterilization.

When the stale medium (or 'solution of x') is added to a fresh medium, we observe a depression of the stopping-point which appears to correspond to the percentage of 'x' which has been added.[3] The lowest depression obtained with a 50 % mixture of perfectly stale medium with fresh medium was 8° C., the stopping-point being 29·5° C.

Parallel with this is the fact that when such graded solutions are inoculated and allowed to stand at a constant temperature, the rate of growth is proportional, as far as macroscopic evidence can be trusted, to the percentage of 'x' which is present,[3] provided that the experiment is not carried on so long as to involve secondary staling of the media.

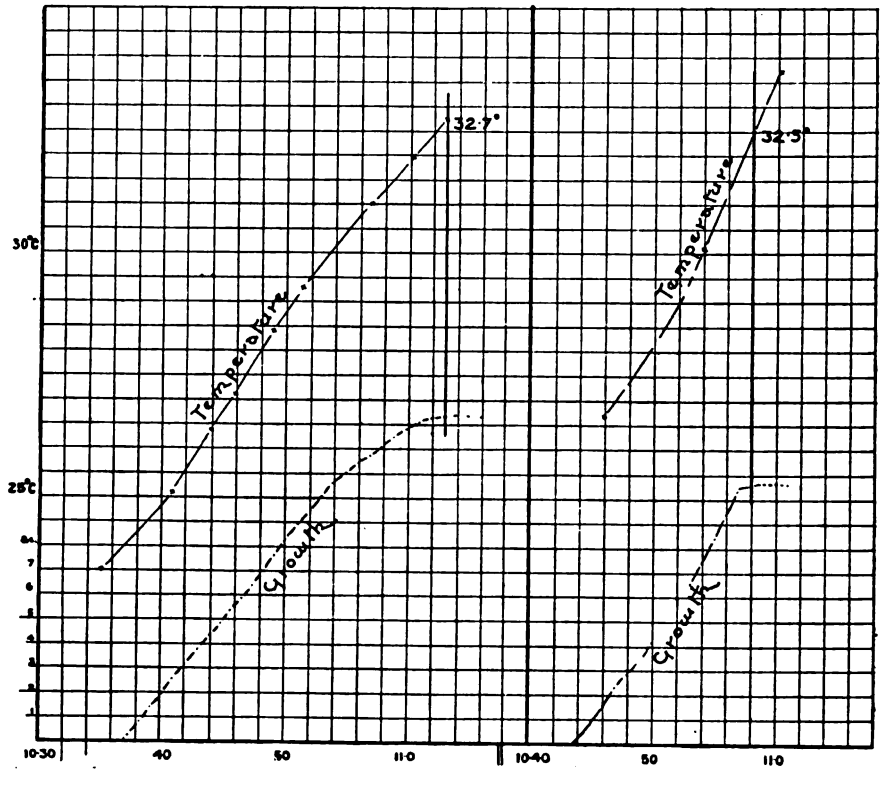
I have not yet been able to show that a proportionate relation exists between the depression of the stopping-point and the amount of 'x' which has been added to the medium. That such a connexion exists is almost beyond doubt, but until its exact nature shall have been determined I consider that the present research is of more interest than value. When we have correlated the influences of the various proportions of 'x' with the

[1] See p. 558. [2] Page 581.

[3] Note also the three growth-gradients in Curve 2.

depressions of the stopping-point we shall be in a position to put a part of the growth process on a chemical footing.

The causes which have prevented this accurate determination are threefold. The body '*x*' has only been obtained in quantities which are relative to the bulk of the solution, whereas it will have to be isolated and handled in definite amount. The slight instability of '*x*' has also to be overcome.



CURVE 4.

Lastly, so much time was spent during the cool months of the Egyptian winter in devising a satisfactory apparatus for the research, that by the time the qualitative experiments had been carried out the summer had arrived.

Decomposition of 'x'.

The first experiments with stale media gave erratic results. The cause of this was traced to the boiling of the media in the steam sterilizer, which partly, or entirely, destroyed the poisonous excreta. This is contrary to the opinion which I formed during the preliminary experiments with mass

cultures, on account of the greater bulk and concentration of the latter, as will be seen below.

The effects of boiling a stale medium are rather erratic. It would seem that the decomposition of '*x*' is more rapid in dilute solution, in thin films, and in small bulk of medium. These facts indicate that it is not an enzyme, and that its disappearance is due rather to chemical change than to physical displacement.

In no case was the stopping-point lowered by boiling the stale medium, although some thirty experiments were made, either directly, or in connexion with some other object. The application of heat to the medium either produced no effect, or else caused growth to cease at a higher temperature than had previously been the case.[1]

DECOMPOSITION OF '*x*'. CURVE 4.

Nearly stale culture medium. Not diluted. Boiled for four hours under reflux condenser, followed next day by two hours' boiling in the steam sterilizer.

Tube inoculated evening of 22/4/07, at 22° C.

Set up 10.15 a.m., 23/4/07. Galvanometer zero, 30 cm.

Readings of micrometer eye-piece scale in length-units of ·003 mm. (columns 2, 3, 4, 5).

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
10 46 p.m.	1	39·0	38·0	·7			
43	1	·7	·7	·6	26·7	33	
44	1	40·3	39·3	·8	26·7	30·5	
49	0·3	40·4	40·1		26·7	28	
50							
51							
10.52 p.m.	·0	45·5	42·5	—	28·5	27	
53	2·0	45·5	43·5	1·0	—	—	
54	—	—	—	—	30·3	31·5	
55	20·0	47	27	—	—	—	
56	18·0	46	28	1·0	33·6	46·5	
57	—	46·8	28·8	·8	—	41·5	
58	—	48·0	30·0	1·2	33·5	37	
59	19·0	49·0	30·0	0·0	—	33	
11·0	15·0	45·0	30·0	—	—	28·5	
A rapidly growing hypha, branching from the one previously under observation.							
11.1 p.m.	12	36	24	—	—	—	
2	14	38	24	0·0	35·0	31·5	
3	18	42	24	—	34·9	27	
4	19	43	—	—	—	—	

In this connexion it may be pointed out that whereas fresh media either give the same stopping-point in duplex curves (Curve 2), or else a depression due to staling, the artificially staled cultures commonly show an increase in the stopping-point when duplicated; in one case it rose from 31·0° C. to 33·5° C. and in another from 29·5° C. to 37·0° C. This would seem to indicate that in the thin film of solution in the damp chamber the body '*x*' is even more readily decomposed than in the large tubes. Further, it is possible that some conditions which protect it from decomposition exist in the cells of the organism.

The series of results given below is typical of the data obtained, and demonstrates all the important points.

Medium from a large flask culture, almost stale; transferred to a series of tubes, and heated at 100° C. in the steam sterilizer for thirty minutes. This solution was then treated in various ways.

(*a*) Inoculated and tested—growth almost negligible, too slight to enable any determination of the stopping-point to be made. In the tube it grew about five millimetres in ten days.

(*b*) Boiled for four hours under a reflux condenser, and for another hour in the sterilizer; growth of fungus free, with a stopping-point at 32·5° C. Curve 4.[1]

(*c*) Mixed with own bulk of water, i.e. 50 per cent. solution.

Tested directly	29.5° C.
Boiled in sterilizer for half an hour . .	35·0° C.
Boiled for another half hour	38·0° C.

(*d*) Diluted with water to 25 per cent.

Tested directly	31·0° C.
Boiled for half an hour	33·5° C.
Boiled for two hours	37·4° C.

[1] Page 580.

DECOMPOSITION OF '*x*'. CURVE 5 *a*.

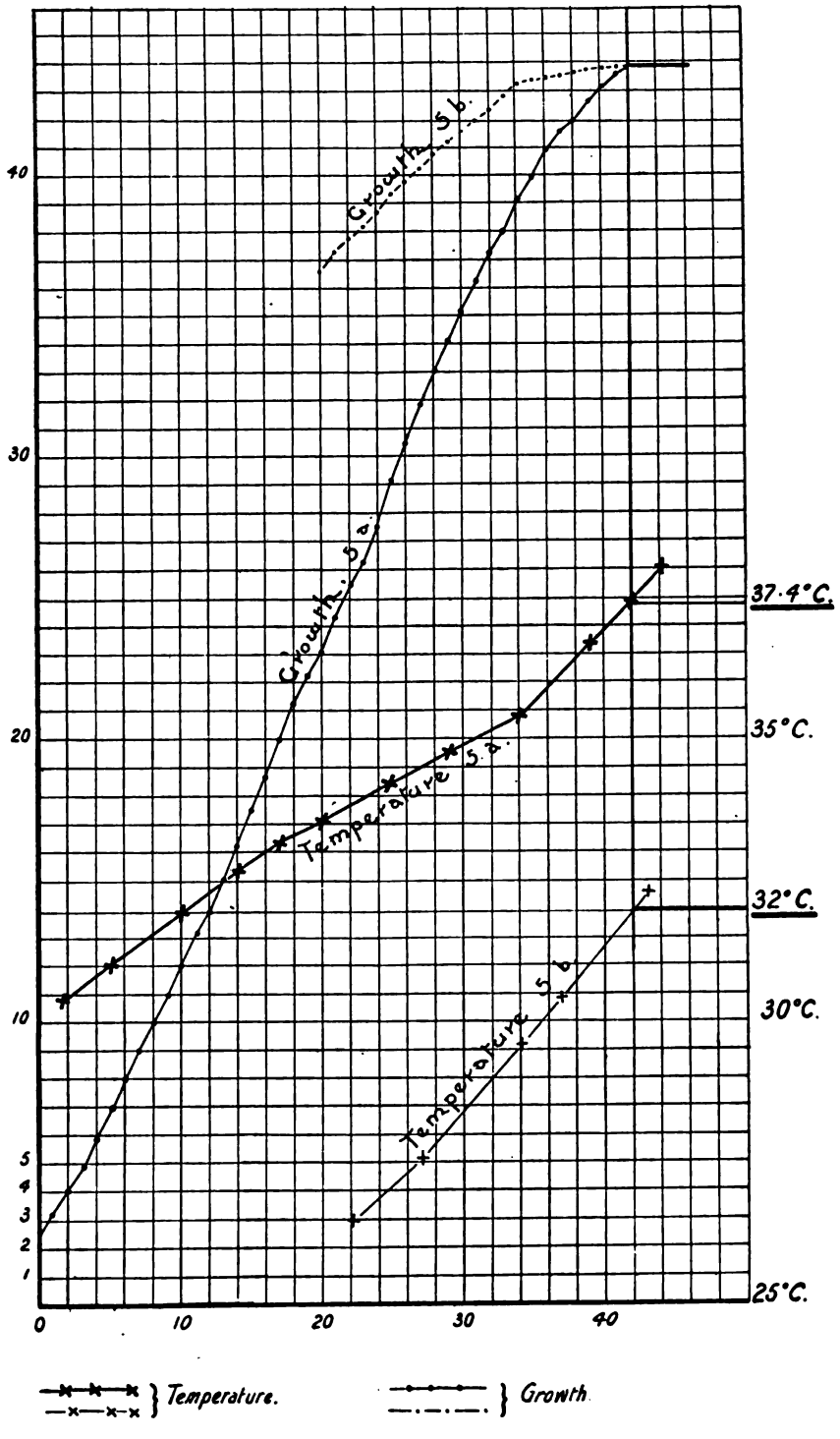
Fifty per cent. fresh Salomonson medium, mixed with 50 per cent. stale ditto. Companion experiment to 5 *b*, but the mixed medium placed in steam sterilizer at 10.30 a.m., boiling by 11.30 a.m., and removed at 1 p.m.

Tube inoculated noon, 11/4/07. Stored at 24° C.

Set up 10 a.m., 12/4/07. Galvanometer zero, 30 cm.

Readings of micrometer eye-piece scale in length-units of ·003 mm. (columns 2, 3, 4, 5).

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
10·30 a.m.	7·0	30·5	23·5	—	30·4° C.	33	
31	8·0	32·2	24·2	·7	·4	31	
32	9·0	34·0	25·0	·8	·3	29	
33	—	·8	·8	·8	—	—	
34	—	—	—	—	—	—	
35	6·3	34·2	27·9	—	31·1	31	
36	—	35·3	29·0	1·1	·1	28	
37	—	36·3	30·0	1·0	—	—	
38	—	37·3	30·9	·9	31·9	33·5	
39	6·7	38·3	31·6	·6	—	31·5	
40	—	39·2	32·5	·9	·8	29	
41	—	40·3	33·6	1·1	—	—	
42	—	41·4	34·7	1·1	32·8	34	
43	7·7	43·5	35·8	1·1	—	32·5	
44	7·0	44·0	37·0	1·2	·7	30	
45	31·0	44·5	13·5	—	—	—	New control, old one indefinable.
46	—	45·7	14·7	1·2	33·3	32	
47	—	47·0	16·0	1·3	—	30·5	
48	28·7	48·2	19·5	—	—	—	New control.
49	—	49·2	20·5	1·0	33·5	32·5	
50	—	50·0	21·3	·8	—	30	
51	24·0	34·3	10·3	—	—	—	New control.
52	—	35·5	11·5	1·2	34·3	37	
53	—	36·3	12·3	·8	—	35	
54	—	37·6	13·6	1·3	·2	32	
55	23·7	38·9	15·2	1·6	—	30	Lamp closer.
56	23·5	40·0	16·5	1·3	—	—	
57	23·3	41·2	17·9	1·4	34·9	34	
58	23·1	42·2	19·1	1·2	·9	32·5	
59	23·0	43·2	20·2	1·2	·8	31	
11.0	23·5	44·7	21·2	1·0	·8	28·5	
1	—	45·8	22·3	1·1	—	—	
2	—	46·8	23·3	1·0	35·7	34	
3	24·0	48·0	24·0	·7	·5	32	
4	23·5	48·7	25·2	1·2	·4	29·5	
5	23·2	49·2	26·0	·8	—	—	
6	15·0	42·0	27·0	1·0	36·9	37·5	
7	—	·6	·6	·6	·8	35·5	
8	—	43·0	28·0	·4	·7	32	
9	—	43·7	·7	·7	36·6	29	
10	14·8	44·0	29·2	·5	—	—	
11	·6	·3	·7	·5	37·4	31·5	
12	15·0	45·0	30·0	·3	·3	29	
13	—	—	—	—	—	—	
14	—	—	—	—	38·0	30	
15	—	—	—	—	—	—	
16	—	—	—	—	—	—	



CURVES 5 *a* and 5 *b*. Two experiments with same medium.

CURVE 5 *b*.

Fifty per cent. fresh Salomonson medium, mixed with 50 per cent. stale ditto. Companion experiment to 5 *a*, but not boiled.

Tube inoculated, 7/4/07. Stored at 24° C.

Set up, 10 a.m., 8/4/07.

1 *Time.*	2 *Control Point.*	3 *End of Hypha.*	4 *Length under Observation.*	5 *Growth per Minute (by difference).*	6 *Temperature of Bath.*	7 *Reading of Galvanometer.*	8 *Notes.*
11·2 p.m.	6·3	30·3	24·0	—	26·5° C.	32	Lamp in.
3	7·1	32·0	24·9	·9	—	32·5	
4	—	32·3	25·2	·3	—	30·5	Apparatus shaken by traffic outside.
5	7·0	32·6	25·6	·4	36·4	29	
6	6·8	32·9	26·1	·5	—	—	
7	4·3	31·1	26·8	·7	27·7	37	
8	4·1	31·3	27·2	·4	—	33	
9	—	·8	·7	·5	—	31	
10	4·0	32·2	28·2	·5	—	29·5	
11	3·9	32·5	·6	·4	—	—	
12	—	—	—	—	28·8?	31	
13	3·8	33·2	29·4	—	—	—	
14	4·5	34·3	29·8	·4	29·6	36	
15	·2	·5	30·3	·5	—	33·5	
16	4·0	34·7	30·7	·4	—	30·5	
17	—	·8	·8	·1	—	—	
18	—	·9	·9	·1	30·4	32	
19	3·8	34·8	31·0	·1	—	28·5	
20	4·1	35·2	·1	·1	—	—	
21	4·0	35·2	·2	·1	31·5	34	
22	—	·3?	—	—	—	31·5	
23	—	·3	·3	·05	—	29	
24	—	·4	·4	·1	—	—	
25	—	—	—	—	32·3	31	
26	—	—	—	—	—	28·5	
27	—	—	—	—	—	—	
28	—	—	—	—	—	—	
29	—	—	—	—	—	—	
30	—	—	—	—	—	—	

THEORETICAL ASPECTS AND CONCLUSIONS.

The Components of the Growth-curve.

A growth-curve has been constructed (Curve 6) to simulate the experimental results, and to show, by analysis, the lines along which further work might be attempted.

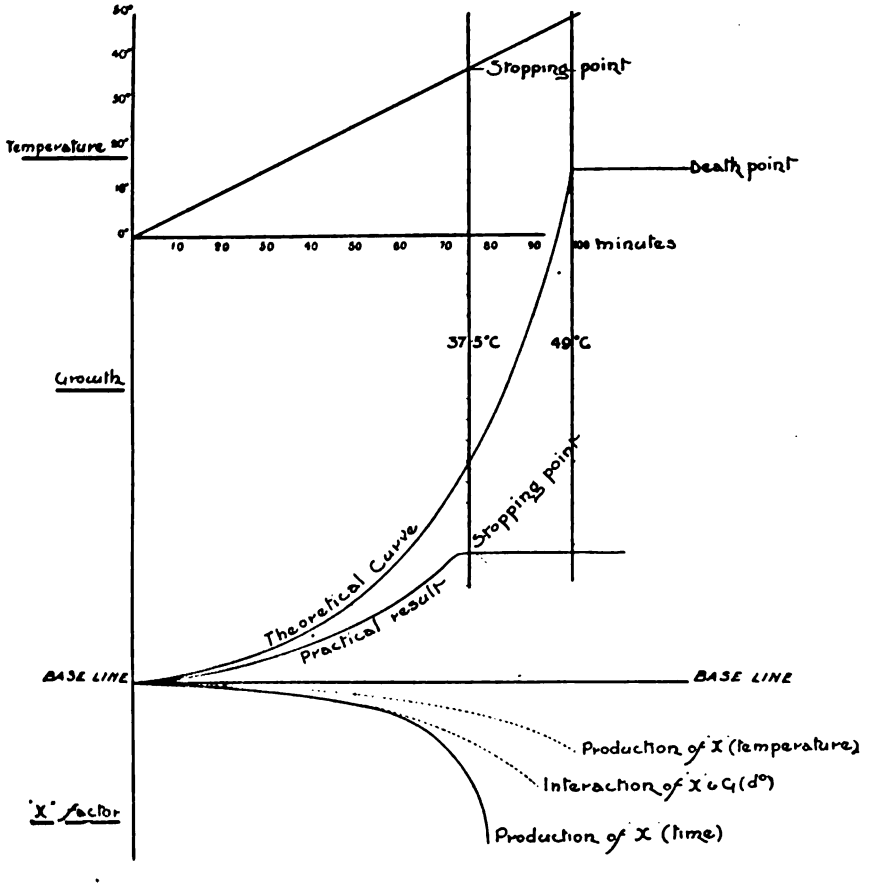
Two special assumptions will be made of which no accurate proof exists, viz., that—

(*a*) The depression of the stopping-point is proportional, in some kind of progression, to the amount of 'x' which is present in the cell at the commencement of the experiment.

(*b*) The decrease in growth-rate at constant temperature is also proportional to the amount of '*x*' which is present in the cell.

Until these assumptions have been proved or disproved the following presentment has no direct value.

Let us start by imagining that some substance, or group of substances (probably situate in the nucleus) is, as it were, the governor which controls



CURVE 6. The components of the curve of growth. Varying temperature.

the constructive metabolism of the cell. For convenience in discussion we will again have recourse to a symbol, and denote this governor by *G*.

We know that the body '*x*' is excreted as a result of the growth-process, and that its excretion ceases with the cessation of growth, and that it exerts an injurious effect upon the process—that is to say, upon *G*. This effect is presumably chemical, and will be affected by rise of temperature.

We also know that 'x' is excreted with greater rapidity at high temperatures, so we will further assume that both these chemical reactions follow Van 't Hoff's law.

Let us now apply this mass of assumptions to the modification of the theoretical growth-curve which is represented above the base line (upper curve).[1] The practical curve is also shown above the base line (lower curve). The difference between these curves represents the antagonistic force exerted by the action of 'x' upon G; this difference is drawn below the base line.

Our object is to find what force, or combination of forces, will simulate this antagonistic curve.

Take first the accumulation of 'x' with rise of temperature. This will give us a curve below the base line, of less altitude than the theoretical growth-curve, but otherwise similar. Consequently, it can never neutralize the latter, but can only depress it, without even altering its form.

The same applies to a curve which shall represent the action of 'x' upon G.

As yet we have only taken into account the rise in temperature, and have disregarded the fact that time is also increasing. Now, the production of 'x' from the protoplasm is obviously a slow reaction; consequently, its rate of accumulation in the cell will be represented by a curve showing an arithmetical progression at constant temperature, and by the combination of this with the previous curve when the temperature is rising.

It is possible that other time-curves may be involved, such as one representing the mass-reaction between 'x' and G.

The results of three such antagonistic curves acting on the theoretical growth-curve are, however, not unlike the results obtained in practice.

Another curve is appended,[2] based on the same assumptions, in order to analyse the effects of a constant high temperature. The rates of acceleration have, of course, been deliberately adjusted.

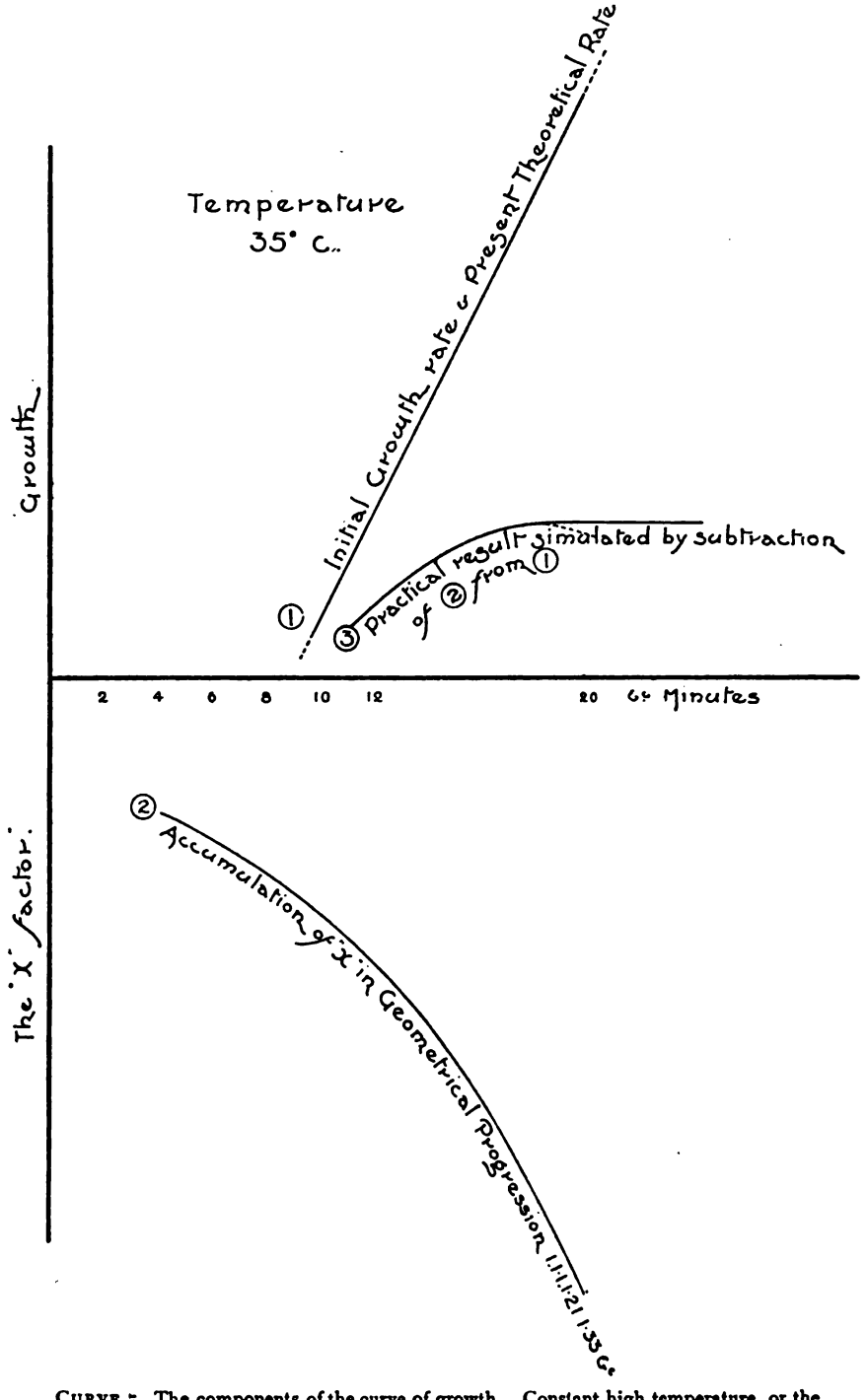
With regard to the manner in which these antagonistic forces might be determined:—

It is clear that the first data will be given by the stopping-point depressions effected by solutions of known concentration of 'x', and the remainder by comparison of different rates of heating. Lastly, if 'x' can be isolated (and this should in any case be the next step in the work) we might be able to determine something as to the chemical nature of G.

There are three apparent objections to this scheme of attack:—

It may not be possible to isolate 'x', which seems to be a somewhat unstable substance; possibly a substitute might be found. The heating apparatus will have to be under much better control than it has been in my experiments. Lastly, we are confronted with a doubt as to whether

[1] Curve 6. [2] Curve 7.


Temperature
35° C.
Growth
Initial Growth rate & Present Theoretical Rate
① Practical result simulated by subtraction of ② from ①
③
2 4 6 8 10 12 20 &c Minutes
The 'X' factor.
② Accumulation of 'X' in Geometrical Progression 1.1.1.2.1.33 &c

CURVE 7. The components of the curve of growth. Constant high temperature, or the Time Factor.

the slight irregularities in the stopping-point, noticed under conditions which are as uniform as I have been able to devise, may not be enough to prevent accurate determinations; some other organism might be employed if this were found to be the case.

The Time Factor in other Life-processes.

This proof of the concrete and chemical nature of the time factor in growth leads one to hope that the same may be found to apply to the time factor in assimilation and respiration. From this point of view a fact recorded by Blackman might be suitable for further investigation; namely, the low assimilatory maximum shown by Cherry Laurel leaves in their second year, as compared with the result which they give in their first year. If this depression of the maximum were due to the accumulation of a specific inhibitory body in the leaf cells, it should be possible to extract this body from two-year-olds, and supply it to the young leaves; when it entered the cells of the latter their assimilatory maximum should at once fall.

If, on the other hand, such treatment produced no effect, there would still be the possibility that this body really existed, but that the young leaves had the power of decomposing it, and that this power was lost in age.

General Conclusions.

1. The growth rate at various temperatures accords with the expectations of Van 't Hoff's law.

2. The decrease, and ultimate cessation of growth at high temperatures, is due to the accumulation of katabolic products in the cells.

3. This cessation is distinct from the disorganization of the protoplasm by heat, which results on a further rise of temperature to the death-point.

4. The same products are formed at low, as at high temperatures, but with greater rapidity in the latter case.

5. To the rapid formation of these bodies is due the injurious effect of prolonged exposure to sub-maximal temperatures, commonly known as the time factor. This time factor is identical with the phenomenon of 'staleness' in rich cultures of fungi.

6. In the case of isolated cells these bodies diffuse out into the surrounding liquid. In multicellular organisms they have to be otherwise disposed of, probably by decomposition; since the conditions under which this decomposition takes place must be fairly uniform in the interior of a higher plant, these latter show in consequence a well-marked 'Optimum', which is the expression of the internal struggle between the increasing rapidity of chemical change with rise of temperature, and the inhibiting effects of the accumulating katabolic products.

Practical Conclusions.

7. Under the conditions of this research it is unlikely that any other factor should be limiting at the 'stopping-point' than the factor 'x'.

8. The Pfeiffer Hot-Box is quite unreliable for accurate measurements of the varying temperature of objects in the microscope field.

9. The ordinary forms of hanging-drop culture chamber are also unreliable when used with other than constant temperatures, on account of the irregular heating of the various parts, of the temperature-lag induced by the proximity of the objective, and of the changes in drop-concentration which result from this irregular heating.

My sincere thanks are due to Dr. F. F. Blackman, who has given me much assistance in the early stages of the work by advice on apparatus and methods. The present paper is to be regarded as an attempt to extend to growth-phenomena the principles which he has outlined in 'Optima and Limiting Factors'.

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Appendix.

Influence of Temperature on Infection.

The artificial infection of a cotton seedling with the Sore-shin fungus can be readily effected by placing the former on several layers of damp blotting-paper in a Petri dish, and then placing a fragment of rapidly growing mycelium from a cool culture in contact with it. The dish is then stored at 20° C., and within twenty-four hours the seedling will be found to have rotted at the point of inoculation. The portion of the seedling stem which is in contact with the damp paper is not usually rotted, because at that point the aeration is usually insufficient.

If the trial is repeated at a temperature of 33° C., a brown superficial scar is formed,[1] but the fungus does not extend its attack into the inner layers of the cortex.

At 38° C. no infection can be found.

On the 'x-hypothesis' it should be possible to bring about rapid infection at the higher temperatures by keeping the infected area in contact

[1] The whole research was founded on this observation.

with water, so as to wash away the body '*x*' before it had time to accumulate in such amount as to inhibit the growth of the fungus through the cells of the host. This can actually be done by placing the seedling in the bottom of a Petri dish and adding enough water to submerge all but the upper third of the horizontal stem. If it is then inoculated, and left for a day at as high a temperature as 35° C., the following appearances are observed.

Under water:	No infection, because aeration is insufficient.
In the air:	Slight superficial brown scar.
On the water-level:	Deep brown rotten patch, which follows the line of equilibrium between the two former conditions.

Traumatic Ray-Tracheids in Cunninghamia sinensis.[1]

BY

EDWARD C. JEFFREY,

Professor of Plant Morphology, Harvard University.

With Plate XXXI.

IT is usual at the present time among perhaps the greater number of botanists to regard the Coniferales as an ascending series, beginning with the Taxineae, as relatively the most primitive, and passing through the Cupressineae and Taxodineae to the Abietineae, the most highly specialized and modern. This view is based on the greater simplicity of structure of the first-named tribe and the more complex organization of the latter. Yet there is practically nothing in the palaeobotanical evidence which really gives it support. Moreover, it is beginning to be realized that apparent simplicity of structure is by no means a reliable criterion of greater antiquity. In fact, quite the opposite conclusion more often results from the consideration of the extinct older forms. For example, the Calamites were infinitely more complicated in both their reproductive and vegetative structures than is their living survivor, the genus *Equisetum*. Similarly, the organization of the Lepidodendroids was very much more elaborate than is that of any of the existing representatives of the Lycopodinean stock.

The study of reversions, especially of those which are susceptible of experimental treatment, has become of increasing importance in morphology, for if in a presumably more ancient, because more simply organized group, it can be shown that reversions to a type of structure possessed by a more complicated related group may be experimentally produced, it becomes at once extremely probable that the more highly organized forms are the more ancient. This conclusion becomes a certainty if it is supported by a considerable body of collateral evidence, especially if such evidence is derived from the study of extinct forms.

The complicated structure of the medullary rays in the existing Abietineae, composed of both parenchyma-cells and so-called ray-tracheids, as well as the very elaborate system of ligneous resin-canals found in the tribe, are considered by many as evidence of extreme specialization and

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 13.

[Annals of Botany, Vol. XXII. No. LXXXVIII. October, 1908.]

consequent modernity. This complexity of organization is found also in the reproductive organs of the group. Penhallow has called attention, however (Anatomy of North American Coniferales, American Naturalist, vol. xxxviii, p. 341, 1904), to the presence of the characteristic marginal ray-tracheids of the Abietineae in the Cupressineous genera, *Juniperus*, *Cupressus*, and *Thuya*. They occur in the following species in a sporadic and uncertain manner, *Juniperus nana*, *Cupressus thuyoides*, and *C. nootkatensis*, and in *Thuya japonica*. They are also found in certain of the Taxoidineae. In his Comparative Anatomy (Eng. Trans., p. 490) De Bary describes their presence in the monotypic genus *Sciadopitys*. More recently Gothan (Just Jarhresberichte, vol. 31, p. 848), in a review of a work by the present author on the genus *Sequoia*, mentions the sporadic occurrence of marginal tracheids in the old wood of *Sequoia gigantea*. Finally the writer has observed the presence of marginal tracheids in the wood of *Cunninghamia sinensis*, under conditions to be described below. It will appear from the above that both in those genera which at present it is customary to include under the Cupressineae, and in those which are united under the Taxodineae, there are somewhat numerous instances of the occasional occurrence of marginal ray-tracheids, such as are a characteristic feature of the living Abietineae. The genus *Cunninghamia*, as will be shown below, is of particular interest in this connexion, because it apparently offers the first case in which the conditions affecting the appearance of marginal tracheids have been elucidated in the case of the Cupressineae and Taxodineae.

Figure 1, Plate XXXI, represents a transverse section of the wood of *Cunninghamia sinensis* under a moderate degree of magnification. The affinities of the wood are easily inferred from the considerable number of resin-cells, appearing as dark dots among the tracheids, and the absence of resin-canals, such as are found in those Abietineae which more nearly resemble *Pinus* in their internal organization. It is the view of Professor Penhallow that the resin-canals of such forms as *Pinus* have been derived from a concentration of resin-cells, such as are found in *Cunninghamia* and other Cupressineous and Taxodineous genera. That this view can scarcely be maintained will be shown at a later stage. Attention was first drawn to the presence of ray-tracheids in *Cunninghamia* in longitudinal radial sections of a branch fourteen years old and rather over a centimetre in thickness. The segment of the branch was between two and three centimetres in length, and presented no unusual features in the first eleven years of growth. In the last three years, however, there occurred here and there rays, characterized by the presence of marginal tracheids. Not more than two such rays were seen in any section, but in the considerable number of sections examined they showed themselves as distributed throughout the entire length of the piece of wood. In one case marginal tracheids were found on both upper and lower sides of the ray, but in the other instances

they were present on one side only, and that generally the lower one, as inferred from the outgoing leaf traces. Fig. 2, Plate XXXI, shows one of the rays with marginal tracheids under a moderate degree of magnification. The ray in question passes through three annual rings, of which the innermost (on the left of the figure) is considerably narrower than the remaining two. In this example the marginal tracheids appear on one side of the ray only, and are confined to the two internal annual rings. In other cases marginal tracheids have been found in the outer ring as well. Fig. 3, Plate XXXI, shows a portion of the same ray illustrated in the last figure, covering the median annual ring, more highly magnified. It is now possible to distinguish clearly that the marginal ray-cells present characteristic features on one side of the ray, in the absence of the protoplasm found in the remaining cells of the ray, and their relation to the tracheids of the wood by typical bordered pits, smaller in size than those which connect tracheid with tracheid. Fig. 4, Plate XXXI, shows a part of Fig. 3, still more highly magnified. In this figure it is possible not only to make out the connexion of the marginal ray-tracheids with the tracheids of the wood by means of typical bordered pits, but also the fact that the ray-tracheids are related to the protoplasmic ray-cells along their lower horizontal wall by unilateral bordered pits similar to those found in the horizontal walls of ray-tracheids in *Pinus*, where they come in contact with parenchymatous ray-cells. In only one or two instances have bordered pits been found in the terminal walls connecting the ray-tracheids with one another. Generally these walls are entirely free from pitting. The ray-tracheids of *Cunninghamia* resemble those described by Penhallow in *Cupressus*, *Juniperus*, and *Thuya* in being without the dentate projections characteristic of the Hard Pines, and found also by De Bary in the case of *Sciadopitys* (loc. cit.).

The discovery of ray-tracheids in the wood of the branch of *Cunninghamia* under discussion led to the examination of the rest of the woody cylinder for the same phenomenon. It was found that the rays, with tracheids, occurred in only about half of the circumference of the stem, and were quite absent in the remainder. The half of the woody cylinder without the rays possessing ray-tracheids showed clearly the presence of wound-cap, consisting of three years' growth and beginning inwardly with a typical wound-callus. Fig. 5, Plate XXXI, shows a longitudinal section through this region. Near the middle of the section is a dark stripe, representing the wound-callus. On the left of this is the normal wood formed previously to injury. On the right is the wound-cap, consisting of three years' growth, terminating outwardly in the phloem, which appears as a dark stripe. It is to be noted that the wound-cap is characterized by a considerable increase in the number of medullary rays present over that found in the normal wood. Fig. 6, Plate XXXI, shows a more magnified image of the radial

section of the wound-cap. The very numerous rays are readily distinguished as dark transverse stripes. It is possible to make out that none of those shown in the figure is provided with ray-tracheids. The amount of wood parenchyma is also abnormally large in the region of the wound-cap. Sections from the opposite side of the stem are characterized by a more normal amount of wood parenchyma and a normal number of medullary rays. The latter in some instances, as has been described above, show the presence of ray-tracheids. A careful examination of this and other fragments of wood has failed to reveal the presence of ray-tracheids, except in the region representing the annual rings formed after injury and in the part of the circumference of the wood more remote from the actual wound.

It appears from the account given in the paragraphs above that in the genus *Cunninghamia* there are found, in definite relation to injury, marginal tracheids in the rays such as are typical of the Abietineae. It would perhaps be possible to regard these as a mere abnormality did they not also occur in other representatives of the Cupressineae and Taxodineae. Their presence is apparently significant. In the Abietineous genera, *Pinus*, *Picea*, *Pseudotsuga*, and *Larix*, not only are marginal tracheids a characteristic feature of the rays of the normal wood, but ligneous resin-canals likewise occur normally. In the Abietineous genera, *Cedrus*, *Tsuga*, *Abies*, and *Pseudolarix*, ray-tracheids are normally present in only the two first named genera. Resin-canals are entirely absent in all four in the mature wood, *except as the constant accompaniment of injury. In the first year's growth of the root, and in a few cases in the cone axis and in the first year's growth of vegetative branches*, resin-canals occur normally in the four last-mentioned Abietineous genera. It is a reasonable conclusion on ordinary biological principles to consider, on the basis of the evidence of the living Abietineae alone, that the presence of ligneous resin-canals is ancestral for that tribe, and that the normal occurrence of resin-canals in the first annual ring only, as a result of injury to the mature wood, in the genera, *Cedrus*, *Tsuga*, *Abies*, and *Pseudolarix*, represents phenomena of recapitulation and reversion respectively. This view is much strengthened by the consideration of the fossil forms. It has been recently shown by the present writer that there are the best of reasons from the anatomical palaeobotanical side for regarding *Pinus* as the oldest of the living Abietineae, and as related to the archaic genus *Prepinus* (Jeffrey, E. C., Structure of the Leaf in Cretaceous Pines, Ann. Bot., vol. xxii, pp. 207–20, 1908). *Prepinus*, in the anatomical structure of its leaf, a feature recognized on all hands to be most important and significant, shows a remarkable detailed resemblance to certain Cordaitales. In this genus the structure of the wood, so far as the presence of resin-canals is concerned, was identical in structure with living and Cretaceous Pines. Further, Fliche has obtained structural evidence for the existence of *Cedrus*

in the Infracretaceous, this being the oldest well authenticated record of the occurrence of any of the four genera, *Cedrus*, *Tsuga*, *Abies*, and *Pseudolarix* (Fliche, P., Études sur la Flore fossile de l'Argonne, Bull. Soc. Sci. Nancy, 1896). Fliche's observations are all the more significant, because it is precisely the genus *Cedrus* which on anatomical and experimental grounds we should regard as the most ancient of its series, for by its wound reactions it shows a closer relationship to *Pinus* than any of the three allied genera, in that the traumatic resin-canals occur in both the horizontal and vertical planes, instead of in the vertical plane only, as in the remaining three (Jeffrey, E. C., Comparative Anatomy and Phylogeny of the Coniferales, II, Abietineae, Boston Society of Nat. Hist. Memoirs, 1905). It follows both because there are strong reasons for regarding *Pinus* as a very old representative of the Coniferous stock, and because *Cedrus*, *Tsuga*, *Abies*, and *Pseudolarix* show clear indications of descent from ancestors possessing at least the ligneous characteristics of *Pinus*, that there are good reasons for regarding the Abietineae as an ascending series, beginning with more complicatedly organized forms like *Pinus*, and terminating with simplified genera like *Abies* and *Pseudolarix*. The views stated here are in strong opposition to those expressed by Penhallow in his recent works. They seem, however, to rest on sound anatomical and palaeobotanical evidence. Professor Penhallow has not brought forward any good evidence for his view that the ligneous resin-canals of *Pinus* and its allies are derived from aggregations of the resin-parenchyma, which is found in the Taxodineae and Cupressineae. His view is that the resin-canals appear first as imperfect cysts in the wood and afterwards become organized into a perfect system of resin-canals, such as is characteristic of *Pinus*. Professor Penhallow has failed to recognize that his resin-cysts are always of traumatic origin, and are not a normal feature of wood structure as he assumes. Further, if we for the sake of argument were to admit Professor Penhallow's hypothesis, we should encounter the difficulty that the resin-canals not only have to develop from the imperfect cysts, but that these have in the course of evolution to become connected with each other and with the perfect cortical system of resin-canals, since in *Pinus* not only the ligneous canals form a complete system, but these are also in continuity with those found in the inner bark. There is every reason to believe that, in the forms with resin-canals less perfectly developed among the Coniferales, we have rather to do with the isolation of parts of a once complete system as the result of degeneration than the opposite process, as is indicated by Professor Penhallow's hypothesis. Further, not only in the matter of the degeneracy of the ligneous resin-canals are *Pseudolarix* and *Abies* the uppermost terms of the Abietineous series, but also in the loss of the other characteristic feature of the living Abietineae, viz. the marginal tracheids of the medullary rays. In *Pseudolarix* marginal tracheids have quite disappeared, while in

Abies the only well authenticated record of their occurrence is in *A. balsamea* (De Bary, Comparative Anatomy, Eng. Trans., p. 490), a record confirmed by Penhallow (Manual of North American Gymnosperms, pp. 88, 258, Boston, 1907).

After having discussed the evolutionary history of the Abietineae in relation to the development of ligneous resin-canals and the presence of marginal ray-tracheids, a necessary preliminary on account of the clear and definite character of the evidence in this tribe, we are in the position to return to the sporadic marginal tracheids of certain Cupressineae and Taxodineae. Attention has already been called to the observations of De Bary, Penhallow, and Gothan in regard to the occurrence of marginal ray-tracheids in these tribes. Professor Penhallow has suggested that their presence in the cases described by him is the first step, so far as this feature is concerned, in an upward evolution, which ends in the genus *Pinus*. If we add to his observations the recorded presence of marginal tracheids in three Taxodineous genera, we shall have to assume, if we adopt his hypothesis, that this upward evolution has begun independently in six distinct genera. It appears extremely unlikely on general grounds that *Sequoia*, *Sciadopitys*, *Cunninghamia*, *Cupressus*, *Juniperus*, and *Thuya* should each of them independently have varied in a direction leading towards the elaborate wood structure of *Pinus*. It seems more probable and more in accordance with accepted biological principles that the various Taxodineous and Cupressineous genera under discussion have all inherited their ray-tracheids as vestigal characters from a common ancestry. This view of the case is strengthened by a consideration of the mode of occurrence of ligneous resin-canals in the genus *Sequoia*. It has been shown by the present writer (Memoirs Boston Soc. Nat., vol. v, No. 10, 1903) that in *S. gigantea* resin-canals appear normally in the wood of the female cone and in the first years' growth of vigorous vegetative branches and traumatically as a result of injury in the mature wood. In *S. sempervirens* they occur only as the result of wounding. It appears highly probable that *S. gigantea* is the older of the two existing species of the genus. This is rendered probable by the more abundant occurrence of resin-canals in this species, and by the fact that it shows the presence of marginal ray-tracheids which have not yet been found in *S. sempervirens*. The presence of resin-canals in the first annual ring and in the cone-axis of *S. gigantea* would on this interpretation constitute an example of recapitulation, while the traumatic resin-canals which are found in both species, as well as the marginal tracheids of *S. gigantea*, should be regarded as reversions to the ancestral type of wood.

The strongest evidence, however, that neither *Sequoia* nor any other Taxodineous or Cupressineous genus has been the ancestor of the general Abietineous line is furnished by recent discoveries in Mesozoic Botany.

Dr. Arthur Hollick and the present writer have been engaged for some time on a Monograph of the Coniferales from the Lower Cretaceous beds of Kreischerville, Staten Island, N.Y., which is now in the press. The generally accepted view in regard to *Sequoia* is that it appeared geologically at a very early period and antedated *Pinus*. Remains, which have been referred by Zeiller and others to *Sequoia*, from their external features alone, are found as far back as the Jurassic, and in the Cretaceous such fossils become exceedingly numerous. As a result of the study of material with structure preserved, both of leafy twigs and cone-scales, we have come to the conclusion that the forms from the Cretaceous, which have been referred to *Sequoia* as well as to the somewhat similar genus *Geinitzia*, are really not of Taxodineous affinities at all but are Araucarian Conifers. This conclusion has been reached not only because the species in question show no Taxodineous features, but from the much more important consideration that the internal organization both of the twigs and cone-scales is entirely Araucarian. It is significant in this connexion to note that the foliage shoots, referred subsequently to *Sequoia* and *Geinitzia*, were originally put, on account of their habit, under the genus *Araucarites*. Indeed, it would not occur to any one familiar with the foliage shoot of the living *S. gigantea* to refer to it the leafy twigs, with four-sided falcate leaves, of the *Sequoia Reichenbachii* and *Geinitzia* types; the reference in fact seems to have resulted from the discovery of the cones of these Mesozoic forms, which present a superficial, although by no means a detailed resemblance to those of *Sequoia*. We have also shown in the memoir already referred to that a number of other well-known Cretaceous or Mesozoic genera, commonly referred to Taxodineous or Cupressineous affinities, such as *Thuyites*, *Widdringtonites*, *Brachyphyllum*, *Frenelopsis*, Newberry, and in all probability *Voltzia* as well, are in reality Araucarian Conifers, and have no close relationship with any of the modern species which they simulate in habit. There is in fact no structural evidence, in view of these newly discovered facts, for the occurrence of the living *Sequoia* earlier than the Tertiary, since it is only from this level that woods of the true *Sequoia* type have been described by Knowlton, Penhallow, and others. It of course follows, unless there were other better authenticated representatives of the Taxodineae and Cupressineae in the Mesozoic, that neither of these groups could possibly have furnished the ancestry of *Pinus*, which is geologically so very much older. It is true that there are numerous Cupressinoxyla in the upper Mesozoic, but there is no good reason to regard these as having belonged to the Cupressineae or Taxodineae, rather than to the Podocarpineae. We have never found twigs with this type of wood structure, in the Kreischerville deposits, with their leaves attached. This fact rather points to the woods in question having belonged to the Podocarpineae.

Still another fact must be kept in mind in this discussion, viz. that the wood of early Cretaceous Pines had not yet acquired the marginal tracheids which are found in those of late Cretaceous and later epochs (E. C. Jeffrey and M. A. Chrysler, On Cretaceous Pityoxyla, Bot. Gazette, vol. xlii, pp. 1–14). This conclusion follows not only from the study of the wood-structure of Lower Cretaceous Pityoxyla, but also from a consideration of the anatomy of the first year's growth of vegetative branches and of that of the cone-axis of living Pines, where their marginal ray-tracheids are late in appearing (vegetative branches) or are almost entirely unrepresented (cone-axes). It is further significant in this connexion to note that Conwentz has described the very much retarded appearance of the marginal tracheids in the amber-containing Pityoxyla (Late Eocene or Early Oligocene) of the Baltic amber deposits. This author states that the marginal tracheids are quite absent in the earlier annual rings of the branches and only appear at a late stage of growth (Monographie d. Baltischen Bernsteinbäume, p. 53). The primitive absence of marginal ray-tracheids in the genus *Pinus* makes it still more difficult to accept the hypothesis of Penhallow (Anatomy of North American Gymnosperms, Am. Naturalist, vol. xxxviii, pp. 696–708. Manual of N. A. Gymnosperms, pp. 122–71, Boston, 1907), that this genus and its allied genera have come from Cupressineous or Taxodineous ancestors, which have developed ray-tracheids and resin-canals as well. Moreover *Pinus*, or a closely-allied genus, according to the observations of Goeppert (Revision meiner Arbeiten, Bot. Centralblatt, vol. V, p. 405, 1881) and of Professor Penhallow himself (North American Species of Dadoxylon, Trans. Roy. Soc. Canada, ser. 2, vol. vi, 1900), already possessed ligneous resin-canals in the Palaeozoic.

We can in fact only conclude from all the evidence at our disposal, that *Pinus* and its nearer allies are among the oldest of living Conifers, and that its ancestors possessed ligneous resin-canals already in the Upper Palaeozoic and Lower Mesozoic. Further it acquired marginal tracheids in the later Cretaceous long before the true Sequoias or their allies had come into existence. Since the Taxodineae and Cupressineae, according to our most accurate and recent information, appeared long after the Abietineae, it is neither necessary nor logical to consider any sporadic Abietineous features which they may present, such as the normal and traumatic resin-canals of the living species of *Sequoia* or the marginal ray-tracheids of *Sciadopitys*, *Sequoia*, *Cunninghamia*, *Cupressus*, *Juniperus*, and *Thuya* as indicating that the Abietineae have been derived from either of these groups. On the contrary, the rational explanation of these peculiar features of structure in the Taxodineae and Cupressineae appears to be that they are vestigial or reversionary structures, indicating that these tribes have had an Abietineous ancestry. It is clear from the palaeontological evidence that if the two tribes in question came from the Abietineae,

it must have been at the earliest in the very late Cretaceous or the early Tertiary after the Abietineae had developed both ligneous resin-canals and marginal ray-tracheids. The structure of the female cone in the three tribes under discussion quite favours this view. In the Abietineae the megasporangiate cone is made up of pairs of superimposed scales with oppositely oriented fibrovascular systems. The female cones of the Taxodineae and Cupressineae are made up of single scales, but these contain a double system of fibrovascular bundles oriented as are those of the two distinct scales of the Abietineae. The conditions observed suggest naturally that in the Taxodineae and Cupressineae we find the fusion of two organs, which are still free in the more primitive Abietineae. A consideration of the gametophytic structure in the tribes under discussion apparently leads to a concordant conclusion, for we find both the microgametic and megagametic conditions much more simplified in the Taxodineae and Cupressineae than they are in the Abietineae.

SUMMARY.

1. Marginal ray-tracheids, similar to those characteristic of the Abietineae are sometimes present in *Cunninghamia.*

2. In *Cunninghamia* the ray-tracheids are traumatic in their origin and are most numerous in the region of the injured annual rings diametrically opposite to the wound-callus.

3. The ray-tracheids of *Cunninghamia* present a general resemblance to those described by De Bary, Penhallow, and Gothan, in the Taxodineous or Cupressineous genera, *Sciadopitys*, *Sequoia*, *Cupressus*, *Juniperus*, and *Thuya.*

4. The ray-tracheids in the genera mentioned constitute additional evidence for the derivation of the Taxodineae and Cupressineae from the Abietineae, and on this hypothesis are to be regarded as vestigial or reversionary.

5. All the evidence goes to show that the Taxodineae and Cupressineae did not exist before the very end of the Cretaceous or more probably before the beginning of the Tertiary.

It gives me much pleasure to offer my thanks to the Director of the Royal Gardens, Kew, and to my friend Mr. L. A. Boodle of the Jodrell Laboratory, Kew, for material secured there on a visit to England two years ago.

EXPLANATION OF PLATE XXXI.

Illustrating Professor Jeffrey's paper on Traumatic Ray-tracheids in *Cunninghamia sinensis*.

Fig. 1. Transverse section of the wood of *Cunninghamia sinensis*. × 50.

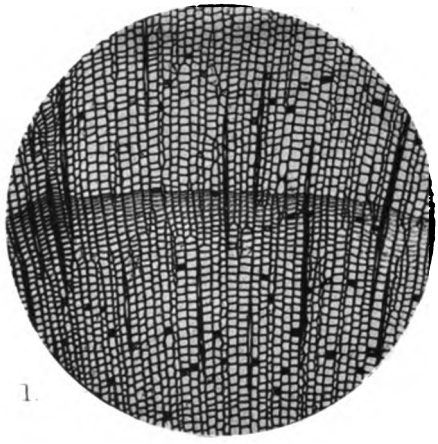
Fig. 2. Longitudinal section through three annual rings of the same, showing a ray with marginal tracheids. × 60.

Fig. 3. Longitudinal view of part of the same section. × 180.

Fig. 4. Longitudinal section of a part of the same more highly magnified. × 500.

Fig. 5. Longitudinal section through wounded region of a branch of *Cunninghamia sinensis*, showing normal wood on the left and wound-cap on the right. × 25.

Fig. 6. Longitudinal section through the wound-cap of the same. × 60.



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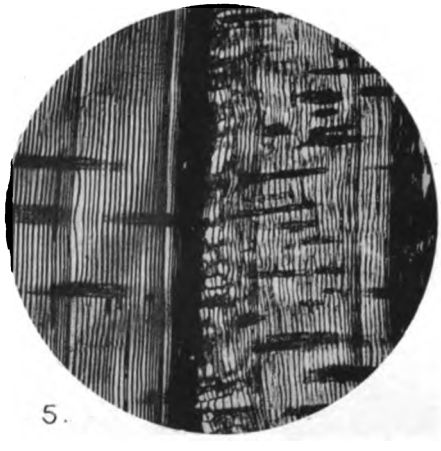

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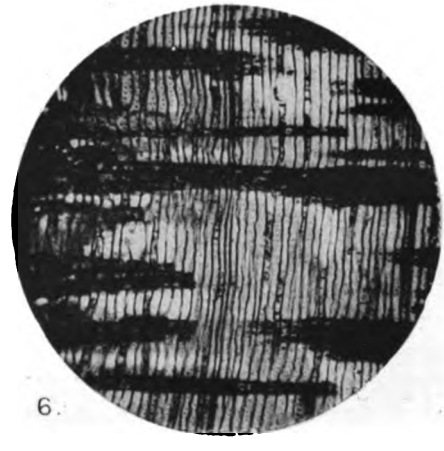

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JEFFREY — CUNNINGHAMIA SINENSIS.

Flowers and Insects in Great Britain.

PART IV.[1]

Observations on the less Specialized Flowers of the Clova Mountains.

BY

J. C. WILLIS, Sc.D.,

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AND

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THIS part of our paper contains all our unpublished observations upon Clova flowers; it will be followed shortly by a concluding part, wherein our results will be reviewed.

CLASS B, § 29. LARGE CRUCIFER TYPE.

148. Cheiranthus Cheiri, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 3 a, 14 a, 33, 34; *Alps* 34.] A garden-plant with homogamous flowers needing insect aid for fertilization, visited in Britain and Germany by Apis and Bombus. Knuth, quoting Schletterer, names a number of other Hymenoptera seen on its flowers in the Tyrol.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., sh. 23. V. 97, 800 ft.

149. Cardamine pratensis, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 a, 14, 18, 24, 25, 34, 40, Hildebrand 1065, de Vries 2460, Warnstorf 2507; *Arct.* 7, 37 a, 38.] Müller, MacLeod, Knuth, de Vries, and Alfken record bees as visiting this plant in North Central Europe; our observations are in singular contrast; for out of 115 insects seen in spring and summer on the flowers,

[1] Part I. Annals of Botany, ix. 1895, p. 227; Part II. xvii. 1903, p. 313; Part III. xvii. 1903, p. 539.

102 were short-tongued flies; and there was not a single bee. The range of the plant extends far north, and its reproduction in Greenland is chiefly asexual by bulbils. The flower is variable in the relative lengths of stamens and pistil, and the probability of spontaneous self-pollination is considerable, Warming thinks very likely in Greenland, but Hildebrand considers the plant in Germany self-sterile.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris napi L., 11–15. VI. 99, 800 ft. Heterocera: *Eriocephalidae*: (2) Eriocephala calthella L., 1–2. VII. 95, 8–1,100 ft. **Diptera.** *Syrphidae*: (3) Melanostoma dubium Ztt., fp. 22. V. 96, 19–2,000 ft. *Empidae*: (4) Empis bilineata Lw., sh. 21. V. 96, 800 ft. *Anthomyiidae*: (5) Trichophthicus sp., fp. 29. VI. 96, 1,300 ft. (6) Anthomyia sulciventris Ztt., fp. 22. V. 96, 19–2,100 ft.; 22–25. V. 97, 7–800 ft. (7 and 8) A. spp., sh. by base in withering flowers and fp. 14–22. V. 96; 19. V. 97; 14. V. 98; 10–15. VI. 99, 8–2,100 ft. *Helomyzidae*: (9) Tephrochlamys sp., fp. 9. V. 98, 800 ft. **Coleoptera.** (10) Meligethes viridescens F., 21. V. 96, 800 ft. (11) Anthobium minutum F., fp. 2. VII. 95, 800 ft.

150. **Arabis caucasica,** Willd. [Lit. *Brit.* 29.] In cultivation. Apis visits it much.

Visitors. **Lepidoptera.** Rhopalocera: (1) Vanessa urticae L., sh. 15–17. IV. 95; 23. V. 97. **Hymenoptera.** Aculeata: *Apidae*: (2) Apis mellifica L., sh. 2–17. IV. 95; 7–15. V. 98. freq. (3) Bombus terrestris L., sh. 15–17. IV. 95. (4) B. lapponicus F., sh. 20. V. 97. **Diptera.** *Muscidae*: (5) Lucilia cornicina F., sh. 16. IV. 95. (6) Pollenia rudis F., sh. 2. IV. 95. *Anthomyiidae*: (7) Anthomyia sulciventris Ztt., 23–24. V. 97. (8) A. sp., fp. 15. V. 98. *Cordyluridae*: (9) Scatophaga sp., sh. 16. IV. 95. All at 800 ft.

151. **Raphanus Raphanistrum,** Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 11, 14, 18, 32, 34, 40, Warnstorf, 2507.] Knuth's autumn observations at Kiel correspond rather closely with Müller's, making it to be visited in Germany by higher insects than at Clova.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., 16. VI. 95. (2) P. napi L., 18. VI. 99. **Hymenoptera.** Aculeata: *Apidae*: (3) Bombus terrestris L., sh. 22. IX. 95. **Diptera.** *Syrphidae*: (4) Eristalis pertinax Scop., 22 IX. 95. (5) Ascia podagrica F., 22. IX. 95. *Muscidae*: (6) Pollenia rudis F., sh. 17–22. IX. 95. *Anthomyiidae*: (7) Anthomyia sp., 17. IX. 95. **Coleoptera.** (8) Meligethes viridescens F., fp. and seeking h. 15–22. IX. 95; 18. VI. 99, very ab. All at 800 ft.

Class B, § 30. Syringa Type.

152. **Syringa vulgaris,** Linn. [Lit. *N.C.E.* 1, 3 c, 14, 34.] In cultivation. *Macroglossa bombyliformis* shows a great preference for its flowers and was recorded as abundant on them in South Scotland in 1899 (Entomologist, 1900, p. 45.)

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris napi L., sh. 12–13. VI. 99.

Heterocera: *Sphingidae*: (2) Macroglossa bombyliformis Esp., sh. a regular visitor, 13–16. VI. 99. *Noctuidae*: (3) Habrostola tripartita Hufn., sh. 12–15. VI. 99. (4) Xylocampa areola Esp., sh. 12–15. VI. 99. (5) Anarta melanopa Thunb., sh. 13. VI. 99. **Diptera.** *Syrphidae*: (6) Syrphus vitripennis Mg., 13. VI. 99. (7) Rhingia campestris Mg., sh. 13. VI. 99. *Empidae*: (8) Empis bilineata Lw., 13. VI. 99. *Anthomyiidae*: (9) Drymia hamata Deg., fp. 13. VI. 99. (10) Hylemyia nigrescens Rnd., 13. VI. 99. (11) Anthomyia sulciventris Ztt., fp. 13–14. VI. 99. **Coleoptera.** (12) Meligethes viridescens F., fairly ab. fp. 10–12. VI. 99. All at 8–900 ft.

Class B, § 31. Mentha Type.

153. Thymus Serpyllum, Linn. [Lit. *Brit.* 23, Marquand 1513; *N.C.E.* 1, 3 c, 12, 14, 14 a, 16, 18, 30, 32, 34, 40; *Arct.* 38; *Alps* 2, 16, 34; *Pyren.* 17.] Gynodioecious. It is well visited by high types of insects.

Visitors. **Lepidoptera.** Rhopalocera: (1) Argynnis aglaia L., sh. 25. VI.–4. VII. 95; 27. VI.–1. VII. 96, 8–1,700 ft. (2) A. selene Schiff., sh. 27. VI. 95, 1,000 ft. (3) Coenonympha pamphilus L., sh. 20. VI–6. VII. 95; 2. VII. 96; 8–1,800 ft. (4) Polyommatus phloeas L., 25. VI. 95, 800 ft. (5) Vanessa urticae L., sh. 6–8. VII. 95; 6–10. VII. 96, 800 ft. (6) Lycaena icarus Rott., sh. 6. VII. 95; 27. VI.–10. VII. 96, 8–1,000 ft. (7) Erebia epiphron Kn., sh. 25. VI. 95, 18–2,000 ft. (8) Pieris napi L., 19. VI. 96, 800 ft. Heterocera: *Geometridae*: (9) Xanthorhoe salicata Hüb., sh. 25. VI. 95; 24. VI. 96, 8–900 ft. (10) Psodos trepidaria Tr., sh. 25. VI. 95, 10–1,500 ft. *Crambidae*: (11) Pyrausta alpinalis Schiff., sh. 5. VII. 95, 800 ft. *Tineidae*: (12) Glyphipteryx fuscoviridella Haw., sh. 1. VII. 95, 800 ft. *Pterophoridae*: (13) Pterophorus tetradactylus L., sh. 28. VI. 95, 1,500 ft. **Hymenoptera.** Aculeata: *Apidae*: (14) Apis mellifica L., sh. 11. VII. 96, 800 ft. (15) Bombus terrestris L., sh. 25. VI.–6. VII. 95, 800 ft. (16) B. lapponicus F., sh. 25. VI.–2. VII. 95; 22. VI.–10. VII. 96, 8–2,300 ft. (17) B. pratorum L., sh. 26. VI.–1. VII. 95, 8–900 ft. (18) B. agrorum F., 26. VI. 95, 800 ft. *Vespidae*: (19) Vespa norvegica F., sh. 23. VII. 95, 800 ft. Petiolata tubulifera: *Chrysididae*: (20) Odynerus pictus Curt., sh. 25. VI. 95, 800 ft. Petiolata parasitica: *Ichneumonidae*: (21) Alomyia debellator (Fabr.)? 1. VII. 95, 800 ft. **Diptera.** *Syrphidae*: (22) Platychirus manicatus Mg., sh. 25. VI.–5. VII, 95; 35. VI. 96, 7–800 ft. (23) Melanostoma mellinum L., 25. VI. 95, 800 ft. (24) Sericomyia lappona L., sh. 1–4. VII. 95; 6. VII. 96, 8–1,700 ft. (25) Volucella bombylans L., sh. 5. VII. 95, 800 ft. (26) Eristalis arbustorum L., sh. 1–6. VII. 95, 800 ft. (27) Syritta pipiens L., fp. 23. VII. 95, 800 ft. *Empidae*: (28) Empis tessellata F., sh. 4. VII. 94; 25. VI.–6. VII. 95; 8–1,000 ft. *Sarcophagidae*: (29) Cynomyia mortuorum L., sh. 11. VII. 96, 800 ft. *Muscidae*: (30) Lucilia cornicina F., sh. and fp. 21. VI.–5. VII. 95; 10. VII. 96, 800 ft. (31) Calliphora erythrocephala Mg., sh. 8–17. VII. 95; 25. VI.–11. VII, 96, 8–1,000 ft. (32) C. vomitoria L., sh. 1–21. VII. 95, 8–900 ft. (33) Pollenia rudis F., sh. 25. VI. 96, 700 ft. *Anthomyiidae*: (34) Hyetodesia incana W. ? sh. 22–25. VI. 95; 2. VII. 96, 8–1,300 ft. (35 and 36) H. spp., sh. 4. VII. 94; 25. VI. 95; 4. VII. 95, 18–2,000 ft. (37) Coenosia sp., 1. VII. 95, 800 ft. (38) Spilogaster quadrum F., fp. 23. VII. 95, 800 ft.

154. **Mentha rotundifolia,** Linn. [Lit. *N.C.E.* 21 b.] An escape from cultivation.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus terrestris L., ? sh. **Diptera.** *Sarcophagidae*: (2) Cynomyia mortuorum L., sh. *Muscidae*: (3) Lucilia cornicina F., sh. (4) Calliphora erythrocephala Mg. (5) Pollenia rudis F., fp. *Cordyluridae.* (6) Scatophaga stercoraria L., fp. **Coleoptera.** (7) Meligethes viridescens F. All 20. IX. 95, 900 ft.

Class B, § 32. Linnaea Type.

155. **Linnaea borealis,** Linn. [Lit. *Brit.* 23, 39; *N.C.E.* 1, 3 c, 8, 14, 14 a, 16, 18, 31, 34; *Arct.* 36; *Alps* 2, 9.] The mouth of the bell is 5 mm. in diameter, the length of the bell is 8 mm. The stigma stands 1 mm. beyond the longer stamens and is curved downwards so as to rub on the back of a visiting insect struggling to penetrate the forest of hairs which guard the honey.

Visitors. **Diptera.** *Anthomyiidae*: (1) Trichophthicus hirsutulus Ztt., fp. **Coleoptera.** (2) Meligethes viridescens F. Both at 1,800 ft. 6. VII. 96.

Class B, § 33. Habenaria viridis Type.

156. **Habenaria viridis,** R. Br. [Lit. *Brit.* Darwin 483; *Alps* 2.]

Visitor. **Coleoptera.** (1) Anthophagus alpinus Payk., sh. 1. VII. 96, 2,400 ft

Class B, § 34. Calluna Type.

157. **Calluna vulgaris,** Salisb. [Lit. *Brit.* 23, 34, 39; *N.C.E.* 1, 3 c, 4, 9, 11, 14, 14 a, 16, 18, 24, 25, 33, 34, 40; *Arct.* 36; *Alps* 2; *Pyren.* 17.] The first heath to flower is generally on some wind-swept point high up, where the plant lies twisted close to the ground; then it bursts into blossom all over the district. As we have shown (Lit. 39) it is probably somewhat anemophilous. We have found plants at Clova upwards of thirty years old (Graebner, in Engler's Bot. Jahrbücher, xx. 1895, p. 500, says that it ceases to flower after fifteen years and dies out in about twenty-four years).

Visitors. **Lepidoptera.** Rhopalocera: (1) Lycaena icarus Rott., sh. 22 VII. 95, 800 ft. Heterocera: *Noctuidae*: (2) Hydroecia nictitans Bkh., sh. 17. IX. 95, 700 ft. **Hymenoptera.** Aculeata: *Apidae*: (3) Bombus terrestris L., sh. 14–23. IX. 95, 7–2,900 ft. (4) B. lapponicus F., sh. 30. VI.–8. VII. 96, 20–2,200 ft. (5) B. scrimshiranus Kirby, 14. IX. 95, 2,000 ft. *Formicidae*: (6) Formica fusca Latr., sh. 17. IX. 95, 700 ft. Petiolata parasitica: *Ichneumonidae*: (7) 1 sp., sh. 19. IX. 95, 2,400 ft. **Diptera.** *Syrphidae*: (8) Platychirus sp., sh. 22. VI. 95, 1,000 ft. *Empidae*: (9) Empis ? vernalis Mg., sh. 19. IX. 95, 2,400 ft. *Muscidae*: (10) Lucilia cornicina F., sh. and fp. 14–19. IX. 95, 20–2,400 ft. (11) Pollenia rudis F., sh. and fp. 14–21. IX. 95, 9–2,400 ft. *Anthomyiidae*: (12) Hyetodesia variabilis Fln. 30. VI.

96, 2,300 ft. (13) Trichophthicus sp., fp. 8. VII. 96, 1,400 ft. (14) Anthomyia sp., sh. 21. IX. 95, 1,100 ft. *Cordyluridae*: (15) Scatophaga stercoraria L., sh. 20–21. IX. 95, 11–2,500 ft. **Coleoptera.** (16) Meligethes viridiscens F., fp. 17–21. IX. 95, 7–1,000 ft. **Thysanoptera.** (17) Thrips sp., 19. IX. 95, 2,400 ft.

Class B, § 35. Valerian Type.

158. Valeriana officinalis, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 c, 18, 32, 33, 34; *Arct.* 36; *Alps*, 2, 9, 16; *Pyren.* 17.] We have referred this flower to Class B, not following those who place it in B′. In Germany, Flanders, and the Pyrenees it has been shown to be rather a Syrphid flower. In the Alps butterflies visit it freely.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., sh. 22. VII. 95, 800 ft. Heterocera: *Noctuidae*: (2) Dianthecia cucubali Fues., sh. 26. VI. 96, 800 ft. (3) Noctua brunnea Schiff., 23. VII. 95, 800 ft. (4) N. baja F., 22. VI. 95, 800 ft. (5) N. C-nigrum L., sh. 2. VI. 95, 800 ft. (6) Celaena haworthii Cuc., sh. 6. VII. 95, 800 ft. (7) Charaeas graminis L., sh. 20–23. VII. 95, 800 ft. *Geometridae*: (8) Thera variata Schiff., sh. 6. VII. 95, 800 ft. *Crambidae*: (9) Pyrausta alpinalis Schiff., sh. 6. VII. 95, 800 ft. **Hymenoptera.** Aculeata: *Apidae*: (10) Apis mellifica L., sh. 15–22. VII. 95; 3–11. VII. 96, 800 ft. (11) Bombus pratorum L., sh. 12–23. VII. 95, 7–800 ft. (12) B. terrestris L., sh. 11. VII. 96, 800 ft. Sessiliventres: *Tenthredinidae*: (13) Allantus arcuatus Forst., eating petals 10. VII. 95, 800 ft. Petiolata parasitica: *Ichneumonidae*: (14) Polyrrhembia tenebricosa Grav., 2. VII. 95, 800 ft. (15) 1 sp., 3. VII. 96, 800 ft. **Diptera.** *Syrphidae*: (16) Leucozonia lucorum L., sh. 15. VII. 95, 800 ft. (17) Platychirus manicatus Mg., sh. and fp. 6–20. VII. 95; 3–11. VII. 96, 800 ft. (18) P. peltatus Mg., sh. 22. VII. 95, 800 ft. (19) Syrphus compositarum Verrall, sh. 17. VII. 95, 800 ft. (20) S. ribesii L., fp. 11–20. VII. 95, 800 ft. (21) S. ? balteatus Deg., sh. 15. VII. 95, 800 ft. (22) Volucella bombylans L., sh. 10–22. VII. 95, 800 ft. (23) Eristalis arbustorum L., sh. 4–22. VII. 95; 24. VI.–11. VII. 96, 800 ft. (24) E. rupium sh. and fp. 2–22. VII. 95, 800 ft. (25) Heliophilus pendulus L., sh. 11. VII. 96, 800 ft. (26) Syritta pipiens L., sh. 17. VII. 95, 800 ft. *Empidae*: (27) Empis tessellata F., sh. 4–20. VII. 95; 24. VI.–11. VII. 96, 800 ft. (28) E. bilineata Lw., 30. VI. 95, 700 ft. (29) E. punctata Mg., sh. 17. VII. 95, 800 ft. *Tipulidae*: (30) Tipula paludosa Mg., 22. VII. 95, 800 ft. *Tachinidae*: (31) Siphona geniculata Deg., sh. 10. VII. 95, 800 ft. *Muscidae*: (32) Calliphora vomitoria L., sh. and fp., 17–22. VII. 95, 800 ft. (33) C. erythrocephala Mg., sh. and fp. 20. VII. 95; 11. VII. 96, 800 ft. (34) Pollenia rudis F., sh. and fp. 4–12. VII. 95, 7–800 ft. *Anthomyiidae*: (35) Hyetodesia incana W., sh. and fp. 2–17. VII. 95; 24. VI. 96, 800 ft. (36) H. basalis Ztt., sh. and fp. 2–4. VII. 95, 800 ft. (37) Spilogaster nigrivenis Ztt., fp. 3. VII. 96, 800 ft. (38) Trichophthicus sp., sh. and fp. 17. VII.–95; 24. VI. 96, 800 ft. *Cordyluridae*: (39) Scatophaga stercoraria L., sh. and fp. 20–22. VII. 95, 800 ft. **Coleoptera**: (40) Meligethes viridescens F., sh. 2. VII. 95; 20. VI.–6. VII. 96, 7–1,700 ft. (41) Epuraea aestiva L., sh. 2–6. VII. 95, 800 ft. **Trichoptera**: (42) 1 sp., 22. VII. 95, 800 ft.

Class B, § 36. Polygonum viviparum Type.

159. Polygonum viviparum, Linn. [Lit. *Arct.* 7, 34, 36, 38; *Alps* 2, 9, 16, 21 a, 21 b.] Reproduction is very largely by bulbils.

Visitors. **Lepidoptera.** Heterocera: *Noctuidae*: (1) Eupithecia nanata Hb., ? sh. 27. VI. 95, 800 ft. *Crambidae*: (2) Pyrausta alpinalis Schiff., 4. VII. 95, 2,700 ft. *Tortricidae*: (3) Tortrix sp., sh. 5. VII. 95, 800 ft. (4) Gelechia sp., sh. 24. VI. 95, 800 ft. **Hymenoptera.** Sessiliventres. *Tenthredinidae*: (5) Allantus arcuatus Forst., 1. VII. 95, 800 ft. **Diptera.** *Syrphidae*: (6) Platychirus manicatus Mg., 4. VII. 95, 900 ft. (7) Syrphus tricinctus Fln., sh. 27. VI. 95, 800 ft. (8) Eristalis rupium F., sh. 27. VI. 95, 800 ft. (9) Syritta pipiens L., sh. 17. VI. 95, 800 ft. *Empidae*: (10) Empis tessellata F., 4. VII. 94, 800 ft. *Bibionidae*: (11) Dilophus albipennis Mg., 10. VII. 96, 2,000 ft. *Muscidae*: (12) Pollenia rudis F., 4. VII. 94, 800 ft. *Anthomyiidae*: (13) Hyetodesia incana W., sh. 27. VI.–4. VII. 95, 8–1,800 ft. (14) H. lucorum Fln., 29. VI. 95, 800 ft. (15) H. basalis Ztt., 29. VI. 95, 800 ft. (16) Spilogaster nigrivenis Ztt., fp. 2. VII. 96, 2,300 ft. (17) Drymia hamata Fln., sh. 4. VII. 95, 1,800 ft. (18) Hylemyia nigrescens Rnd., 24. VII. 95, 800 ft. (19) Homalomyia incisurata Ztt., 4. VII. 94, 800 ft. (20 and 21) Anthomyia spp., sh. 22. VI. 95, 1,700 ft.; 6. VII. 96, 2,000 ft. *Cordyluridae*: (22) Scatophaga sp., sh. 4. VII. 95, 1,800 ft. *Sapromyzidae*: (23) Lauxania cylindricornis F., 4. VII. 94, 800 ft. **Hemiptera.** (24) Nabis flavimarginatus, D. & S., 1. VII. 95, 900 ft.

Class B, § 37. Intermediate Rosaceous Type.

160. Prunus Padus, Linn. [Lit. *N.C.E.* 1, 3 b, 33; *Alps* 34.]

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., sh. 21–22. V. 97, 6–800 ft. **Diptera.** *Syrphidae*: (2) Syrphus punctulatus Verrall, sh. 21–22. V. 97, 6–800 ft. *Anthomyiidae*: (3) Anthomyia sulciventris Ztt., 21–22. V. 97, 6–800 ft. **Coleoptera.** (4) Meligethes ? viridescens F., 21. V. 97, 800 ft.

161. Rubus Chamaemorus, Linn. [Lit. *Brit.* 23; *N.C.E.* 21 a, 21 b; *Arct.* 36, 38; *Alps* 34.] About 20 per cent. of the flowers get fully fertilized and set large fruits, the rest get fertilized in various degrees of completeness: frequently only one carpel may form a drupelet. Flies, which visit it, go by preference to the male flowers.

Visitors. **Hymenoptera.** Petiolata parasitica: *Ichneumonidae*: (1) Hemiteles? 16. VI. 95, 2,000 ft. **Diptera.** *Syrphidae*: (2) Melanostoma dubium Ztt., 19. VI. 95, 2,000 ft. (3) Syrphus vitripennis Mg., 13. VI. 99, 2,300 ft. *Empidae*: (4) Empis lucida Ztt., sh. 16. VI. 95; 17. VI. 99, 9–2,800 ft. (5) E. vernalis Mg., 17. VI. 99, 2,800 ft. (6 and 7) E. spp., sh. 11–17. VI. 99, 15–2,800 ft. *Bibionidae*: (8) Scatopse sp., 11. VI. 99, 1,500 ft. *Anthomyiidae*: (9) Hyetodesia incana W., 19. VI. 95, 2,000 ft. (10) Drymia hamata Fln., 19. VI. 95, 2,000 ft. (11) Hylemyia nigrescens Rnd., 11–12. VI. 99, 15–2,500 ft. (12) Trichophthicus sp., 16–19. VI. 95, 18–2,000 ft. (13) Anthomyia radicum L., 19. VI. 95; 16. VI. 99, 20–2,700 ft. (14) A. sulciventris Ztt., 11. VI. 99, 1,500 ft. (15) Azelia triquetra W., 13. VI. 99, 2,300 ft. **Coleo-**

ptera. (16) Meligethes viridescens F., 16–19. VI. 95, 2,000 ft.; 9–11. VI. 99, 15–2,000 ft. (17) M. aeneus F., 19. VI. 95, 2,000 ft. (18) Anthobium torquatum Marsh., 19. VI. 95, 2,000 ft. (19) Anthophagus alpinus Payk., sh. 19. VI. 96, 3,000 ft. **Thysanoptera.** (20) Thrips sp., 11. VI. 99, 1,500 ft.

162. Rubus suberectus, Anders. [Lit. *Brit.* 34, 39; Marquand 1513; *N.C.E.* 1, 3 b, 4, 11, 16, 32, 33, 34, 40; *Alps* 16; *Pyren.* 17.] In *R. suberectus* we would include any or all the *Rubi suberecti*, were there more than one at Clova: for, following Sir Joseph Hooker, it is our endeavour, in *Rubus*, in *Hieracia* and elsewhere, to equalize our species and, as far as possible, neither to give a fictitious value to small variations nor to shirk the difficulty of drawing distinctions by lumping. But in the literature list above given we have quoted all citations of *R. fruticosus*, sensu amplissimo.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., sh. 22. VI. 95. Heterocera: *Eriocephalidae*: (2) Eriocephala calthella L., sh. 30. VI. 95. **Hymenoptera.** Aculeata: *Apidae*: (3) Apis mellifica L., sh. 22–30. VI. 95. (4) Andrena analis Panz., sh. 30. VI. 95. *Vespidae*: (5) Vespa sylvestris Scop., sh. 30. VI. 95. Sessiliventres: *Tenthredinidae*: (6) Allantus arcuatus Forst., sh. 23. VI. 95. **Diptera.** *Syrphidae*: (7) Platychirus manicatus Mg., sh. 22. VI. 95; 22. VI. 96. (8) Syritta pipiens L., 22. VI. 95. *Empidae*: (9) Empis tessellata F., sh. 30. VI. 95. (10) E. punctata Mg., 22. VI. 96. *Anthomyiidae*: (11) Hyetodesia incana W., sh. 22–30. VI. 95; 22. VI. 96. (12) Trichophthicus sp., sh. 30. VI.–5. VII. 95. *Sepsidae*: (13) Sepsis cynipsea L., sh. 22. VI. 96. **Coleoptera.** (14) Byturus tomentosus F., sh. and in cop. 30. VI. 95. **Thysanoptera.** (15) Thrips sp., 30. VI. 95. All at 800 ft.

163. Cotoneaster microphylla, Wall. In cultivation.

Visitors. **Hymenoptera.** Aculeata: *Vespidae*: (1) Vespa norvegica F., sh. 1. VII. 96, 900 ft. freq. **Diptera.** *Syrphidae*: (2) Platychirus manicatus Mg:, 18. VI. 99, 900 ft. (3) Syrphus vitripennis Mg., 15. VI. 99, 900 ft. (4) Heliophilus sp., 15. VI. 99, 900 ft. (5) Syritta pipiens L., 15. VI. 99, 900 ft. *Tachinidae*: (6) Siphona geniculata Deg., 18. VI. 99, 800 ft. *Muscidae*: (7) Calliphora erythrocephala Mg., 18. VI. 99, 900 ft. freq. *Anthomyiidae*: (8) Anthomyia sulciventris Ztt., 18. VI. 99, 800 ft.

164. Saxifraga oppositifolia, Linn. [Lit. *N.C.E.* 21 a; *Arct.* 7, 34, 36, 37 b, 38; *Alps* 2, 21 b.] Protogynous, with self-pollination when the stamens dehisce. The brilliant patches of flowers in early April attract flies, but bees do not visit them. Plenty of fruit is ripened. The flowers of a handsome Pyreneean variety cultivated at Kew show no self-pollination.

Visitors. **Diptera.** *Bibionidae*: (1) Scatopse sp., 21. V. 97, 1,700 ft. *Muscidae*: (2) Lucilia cornicina F., sh. 14. IV. 95, 10–1,200 ft. (3) Pollenia rudis F., sh. 14. IX. 95, 10–1,200 ft. *Anthomyiidae*: (4) Anthomyia sulciventris Ztt., sh. and fp. 21. V. 97, 2,300 ft. (5) A. sp., sh. and fp. 21. V. 97, 17–2,200 ft.. *Helomyzidae*: (6) Tephrochlamys sp., sh. 9. V. 98, 2,300 ft. *Sepsidae*: (7) Sepsis ? cynipsea L.,

sh. 14. IV. 95, 10–1,200 ft. *Phoridae*: (8) Phora sp., sh. 21. V. 97, 2,300 ft. **Coleoptera.** (9) Anthophagus alpinus Payk., sh. 1. VII. 96, 1,900 ft.

165. Ribes nigrum, Linn. [Lit. *N.C.E.* 1, 34; MacLeod, 1472.] In cultivation and as an escape from cultivation.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus terrestris L., sh. 13. V. 98. (2) B. lapponicus F., 16. V. 98. Both at 800 ft.

166. Menyanthes trifoliata, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 8, 9, 14, 18, 34, Warnstorf, 2507; *Arct.* 38.]

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris napi L. **Diptera.** *Empidae*: (2) Rhamphomyia sulcata Fln. **Coleoptera.** (3) Meligethes viridescens F., ? sh. All 18. VI. 99, 800 ft.

Class B, § 38. Large Geranium Type.

167. Geranium sylvaticum, Linn. [Lit. *Brit.* 23, *N.C.E.* 3 b, 21 a, 21 b, 34; *Arct.* 34, 36; *Alps* 2, 21 a, 21 b.] The flowers sleep at night.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., sh. 24. VI. 95, 900 ft. (2) Lycaena icarus Rott., sh. 24. VI. 95, 900 ft. **Hymenoptera.** Aculeata: *Apidae*: (3) Bombus terrestris L., sh. 5. VII. 95, 700 ft. (4) B. lapponicus F., sh. 24. VI. 95, 900 ft. and 6. VII. 96, 2,300 ft. (5) B. agrorum F., sh. 8. VII. 95, 800 ft., and 6. VII. 96, 2,c00 ft. (6) Andrena analis Panz., 11. VII. 96, 700 ft. (7) A. coitana Kirby, sh. 5. VII. 95, 700 ft. Petiolata parasitica: *Chalcididae*: (8) 1 sp., sh. 11. VII. 96, 800 ft. **Diptera.** *Syrphidae*: (9) Platychirus manicatus Mg., sh. 2–8. VII. 95; 25. VI.–11. VII. 96, 7–800 ft. (10) P. peltatus Mg., sh. 22. VI. 96, 2,300 ft. (11) P. clypeatus Mg., sp. 2. VII. 95, 800 ft. (12) Syrphus sp., sh. 25. VI. 95, 800 ft. (13) Rhingia campestris Mg., sh. 24. VI.–8. VII. 95, 8–900 ft. (14) Eristalis arbustorum L., 25. VI. 96, 700 ft. *Empidae*: (15) Empis tessellata F., sh. 5. VII. 95; 29. VI. 96, 7–900 ft. (16) E. punctata Mg., 5. VII. 95, 700 ft. (17) E. bilineata Lw., sh. 16. VI. 99, 800 ft. (18) E. ? vernalis Mg., 8. VII. 95, 800 ft. (19) E. sp., sh. 29. VI. 96, 900 ft. *Bibionidae*: (20) Scatopse ?, 5. VII. 95, 700 ft. *Muscidae*: (21) Stomoxys calcitrans L., sh. 25. VI. 95, 800 ft. *Anthomyiidae*: (22) Hyetodesia incana W., sh. 2. VII. 96, 2,000 ft. (23) H. lucorum Fln., sh. 24. VI. 95, 900 ft. (24) H. basalis Ztt., sh. 24. VI. 95, 900 ft. (25) Limnophora solitaria Ztt., 26. VI. 96, 2,300 ft. (26) Drymia hamata Fln., sh. 26. VI.–2. VII. 96, 20–2,400 ft. (27) Hylemyia nigrescens Rnd., sh. 15–19. VI. 99, 7–900 ft. (28) Anthomyia sp., sh. 4–5. VII. 95, 700 and 2,300 ft.

Class B, § 39. Veronica Type.

168. Veronica Chamaedrys, Linn. [Lit. *Brit.* 23, 34; *N.C.E.* 1, 3 c, 4, 11, 14, 16, 18, 33, 34, 40; *Alps* 2; *Pyren.* 17.]

Visitors. **Lepidoptera.** Heterocera: *Eriocephalidae*: (1) Eriocephala calthella L., 24. VI. 95, 800 ft. **Hymenoptera.** Aculeata: *Apidae*: (2) Andrena analis Panz., sh. 22–23. VI. 95, 8–900 ft. Petiolata parasitica: *Chalcididae*: (3) 1 sp., 15.

VI.–2. VII. 95, 8–1,000 ft. **Diptera.** *Syrphidae*: (4) Platychirus manicatus Mg., sh. and fp. 20. VI.–20. VII. 95, 8–900 ft. fairly freq. (5) Chrysogaster ? hirtella, Loew, sh. 22. VI. 95, 800 ft. (6) Syrphus luniger Mg., sh. 23–26. VI. 95, 8–900 ft. (7) Ascia podagrica F., 20. VI. 95, 800 ft. (8) Rhingia campestris Mg., 28. VI. 95, 800 ft. *Empidae*: (9) Empis tessellata F., sh. 20. VII. 95, 800 ft. *Tachinidae*: (10) Siphona geniculata Deg., sh. 22. VI. 95; 22. VI. 96, 8–900 ft. *Muscidae*: (11) Pollenia rudis F., 5. VII. 95, 700 ft. *Anthomyiidae*: (12) Hyetodesia incana W., 23. VI. 95, 800 ft. (13) Anthomyia sulciventris Ztt., sh. and fp. 25. VI.–12. VII. 95; 27. V. 97, 7–800 ft. (14) A. sp., fp. 12. VII. 95; 10. VI. 99, 7–800 ft. (15) Coenosia sp., sh. 1–6. VII. 95, 800 ft. *Opomyzidae*: (16) Opomyza germinationis L., 20. VI. 95, 800 ft.

169. Veronica serpyllifolia, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 14, 34, Warnstorf 2507.] Abundant, but very little visited.

Visitor. **Thysanoptera.** (1) Thrips sp., 16. IX. 95, 800 ft.

170. Veronica alpina, Linn. [Lit. *N.C.E.* 21 b; *Arct.* 36, 37 c; *Alps* 2, 9, 21 b.] The flower is 6 mm. broad; its tube 1·5 mm. long. Ultimate self-pollination and apparently fertilization occurs. Abundance of seed is set.

Visitor. **Diptera.** (1) A small fly, sh. 22. VI. 95, 2,600 ft.

171. Veronica officinalis, Linn. [Lit. *Brit.* 23, 34; *N.C.E.* 1, 4, 14, 16, 18, 30, 34; *Alps* 2, 9, 16, 21 a, 21 b, 34.]

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Andrena coitana Kirby, 26. VI. 95. **Diptera.** *Syrphidae*: (2) Platychirus manicatus Mg., 26. VI. 95. *Anthomyiidae*: (3) Hylemyia nigrescens Rnd., sh. 21. VI. 95. All at 800 ft.

172. Veronica scutellata, Linn. [Lit. *N.C.E.* 14.]

Visitors. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1) 1 sp. sh. 6. VII. 95. **Diptera.** *Empidae*: (2) Clinoceira bipunctata Hal., 6. VII. 95. *Anthomyiidae*: (3) Hyetodesia variabilis Fln., 1. VII. 95. All at 800 ft.

173. Veronica Beccabunga, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 c, 33, 34, 40.

Visitor. **Diptera.** *Anthomyiidae*: (1) 1 sp., 17. VI. 9, 800 ft.

Class B, § 40. Myosotis Type.

174. Myosotis arvensis, Hoffm. [Lit. *Brit.* 23.]

Visitors. **Diptera.** *Syrphidae*: (1) Melanostoma mellinum L., sh. 22. IX. 95, 800 ft. *Anthomyiidae*: (2) Limnophora sp., sh. and fp. 1. VII. 95; 11. VII. 96, 7–800 ft. **Thysanoptera.** (3) Thrips sp., 11. IX. 95, 800 ft.

175. Myosotis versicolor, Reichb. [Lit. *Brit.* 23; *N.C.E.* 1, 3 c, 18, 34.]

Visitors. **Diptera.** *Syrphidae*: (1) Platychirus manicatus Mg., 15. VI. 99.

(2) P. albimanus F., 16. IX. 95. *Tachinidae*: (3) Siphona geniculata Deg., fp. 16. IX. 95. All at 800 ft.

176. Myosotis repens, D. Don. [Lit. *Brit.* 23; *N.C.E.* 1, **14,** 18, 30, 33.]

Visitors. **Diptera.** *Empidae*: (1) Empis tessellata F., sh. 1. VII. 95; 29. VI. 96, 8–1,300 ft. (2) E. stercorea L., sh. 1. VII. 95, 800 ft. *Dolichopodidae*: (3) Dolichopus sp., fp. 29. VI. 96, 1,800 ft. *Anthomyiidae*: (4) Hyetodesia incana W., sh. 1. VII. 95, 800 ft. (5) H. variabilis Fln., 1. VII. 95, 800 ft. (6) Limnophora sp., 6. VII. 95, 800 ft. (7) Drymia hamata Fln., fp. 6. VII. 95, 800 ft. (8) Trichophthicus sp., sh. 29. VI.–6. VII. 96, 8–1,300 ft. *Chloropidae*: (9) Chlorops sp., 29. VI. 96, 1,300 ft. **Coleoptera.** (10) Meligethes viridescens F., fp. 29. VI. 96, 1,300 ft.

177. Anchusa sempervirens, Linn. [Lit. *N.C.E.* 4.]

Visitor. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus agrorum F., sh. going from flower to flower, 10. VI. 99, 500 ft.

Class B, § 41. Epilobium Type.

178. Epilobium alsinifolium, Vill. [Lit. *N.C.E.* 21 a; *Arct.* 36; *Alps* 2, 21 a.]

Visitor. **Diptera.** *Ephydridae*: (1) Hydrellia griseola Fln., fp. 6. VI. 95, 900 ft.

179. Epilobium montanum, Linn. [Lit. *Brit.* 23; *N.C.E.* 3 b; 9, 14, 18, 21 a; *Alps* 34.]

Visitor. **Diptera.** *Syrphidae*: (1) Platychirus ? manicatus Mg., 5. VII. 96, 1,400 ft.

180. Epilobium angustifolium, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 11, 16, 18, 25, 33, 34, 40; *Arct.* 36, 38; *Alps* 2, 9, 21 b, 34.] One of the Class B which most nearly is a Bee flower. Bombi visit it freely and Apis abundantly in most places. Our Clova observations want amplifying. In Arctic regions self-pollination occurs.

Visitors. **Lepidoptera.** Heterocera: *Noctuidae*: (1) Miana fasciuncula Haw., sh. 30. VI. 96. **Hymenoptera.** Aculeata: *Apidae*: (2) Bombus terrestris L., sh. 11. VII. 96. **Diptera.** *Anthomyiidae*: (3) Hyetodesia basalis Ztt., sh. 30. VI. 96. (4) Anthomyia radicum L., sh. 11. VII. 96. (5 and 6) A. sp., sh. 21. VI.–11. VII. 96. *Sepsidae*: (7) Sepsis cynipsea L., sh. 11. VII. 96. All at 800 ft.

TABLE XXXIII.

Actual number of individuals visiting the flowers of Class B.

	Apis.	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
148. Cheiranthus Cheiri . .	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
149. Cardamine pratensis . .	—	—	—	—	—	—	—	6	—	2	2	102	5	—	117
150. Arabis caucasica . . .	3	1	—	—	—	—	—	1	—	—	—	9	—	—	14
151. Raphanus Raphanistrum	—	3	—	—	—	—	—	2	—	—	2	5	163	—	175
152. Syringa vulgaris . . .	—	—	—	—	—	—	—	25	—	—	5	17	17	—	64
153. Thymus Serpyllum . .	1	22	1	—	1	—	1	44	3	—	24	92	—	—	189
154. Mentha rotundifolia . .	—	1	—	—	—	—	—	—	—	—	—	19	2	—	22
155. Linnaea borealis . . .	—	—	—	—	—	—	—	—	—	—	—	3	1	—	4
156. Habenaria viridis . . .	—	—	—	—	—	—	—	—	—	—	—	—	4	—	4
157. Calluna vulgaris . . .	—	24	—	—	1	5	—	2	—	—	2	21	2	1	58
158. Valeriana officinalis . .	28	6	—	1	2	—	—	12	1	—	76	127	5	7	265
159. Polygonum viviparum .	—	—	—	1	—	—	—	1	6	—	5	29	—	1	43
160. Prunus Padus	2	—	—	—	—	—	—	—	—	—	3	2	1	—	8
161. Rubus Chamaemorus .	—	—	—	—	1	—	—	—	—	—	28	59	11	2	101
162. Rubus suberectus . . .	9	—	1	1	—	—	1	1	—	1	6	18	8	1	47
163. Cotoneaster microphylla	—	—	—	—	—	—	6	—	—	—	4	11	—	—	21
164. Saxifraga oppositifolia .	—	—	—	—	—	—	—	—	—	—	—	19	1	—	20
165. Ribes nigrum	—	3	—	—	—	—	—	—	—	—	—	—	—	—	3
166. Menyanthes trifoliata .	—	—	—	—	—	—	—	2	—	—	—	1	21	—	24
167. Geranium sylvaticum . .	—	7	2	—	1	—	—	2	—	—	42	31	—	—	85
168. Veronica Chamaedrys .	—	—	2	—	30	—	—	—	—	1	20	35	—	—	88
169. Veronica serpyllifolia .	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
170. Veronica alpina . . .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
171. Veronica officinalis . .	—	—	1	—	—	—	—	—	—	—	1	1	—	—	3
172. Veronica scutellata . .	—	—	—	—	1	—	—	—	—	—	—	2	—	—	3
173. Veronica Beccabunga .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
174. Myosotis arvensis . . .	—	—	—	—	—	—	—	—	—	—	1	2	—	3	6
175. Myosotis versicolor . .	—	—	—	—	—	—	—	—	—	—	2	1	—	—	3
176. Myosotis repens . . .	—	—	—	—	—	—	—	—	—	—	5	12	1	—	18
177. Anchusa sempervirens .	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
178. Epilobium alsinifolium .	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
179. Epilobium montanum .	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
180. Epilobium angustifolium	—	1	—	—	—	—	—	1	—	—	—	34	—	—	36
Total	44	69	7	3	37	5	8	99	10	4	229	656	242	16	1429
Percentage	3·08	4·83	·49	·21	2·59	·35	·56	6·93	·70	·29	15·95	45·91	16·93	1·12	

TABLE XXXIV.

Showing that Class B draws more than its share of distinctly desirable insects, and much more than its share of desirable insects.

	Available.		*To Class B.*	
	No.	%	*No.*	%
Distinctly desirable	1,763	10·19	212	14·84
Desirable . . .	1,277	7·37	246	17·21
Indifferent . . .	12,993	75·08	910	63·68
Injurious	1,273	7·36	61	4·26

Class B is found to attract rather more than its share of distinctly desirable insects (15 : 10) and more of the desirable insects (17 : 7). To the distinctly desirable insects the Class proves relatively far less attractive than Class H (*vide* Table XXI in Part III) but relatively more attractive than Class B′ (*vide* Table XI in Part II).

TABLE XXXV.

Insects visiting Class B in different seasons classed by Desirability.

	Spring.		*Summer.*		*Autumn.*	
Decidedly desirable	11	7·63	174	16·08	27	13·30
Desirable . . .	5	3·47	236	21·81	5	2·41
Indifferent . . .	127	88·19	623	57·58	160	78·81
Injurious	1	·69	49	4·53	11	5·41
Total	144		1082		203	

TABLE XXXVI.

Percentages of different groups of insects visiting Class B. in different seasons.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Spring .	4·16	2·77	—	—	—	·69	—	—	3·47	84·02	4·16	·69
Summer	3·51	3·60	·64	3·60	·73	8·96	·82	·36	20·24	45·28	11·18	·92
Autumn		12·31	—	2·95	—	·49	—	—	2·46	22·16	56·65	2·46

TABLE XXXVII.

Desirability of visitors to Flowers of Class B according to colour in percentages.

	Blue lilac.	*Rose-purple.*	*Yellow.*	*White.*	*Green.*
Decidedly desirable	10·17	19·86	3·41	8·45	42·86
Desirable. . . .	20·10	29·43	1·14	23·00	—
Indifferent . . .	61·02	48·19	95·45	65·26	57·12
Injurious	8·71	2·50	—	3·28	—
Total no. of insects	413	720	176	213	7

TABLE XXXVIII.

Percentages of different groups of insects visiting flowers of different colours in Class B.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Blue-lilac . .	—	2·18	·97	7·76	—	8·00	—	·72	18·83	54·36	6·06	·97
Rose-purple . .	4·66	8·53	·32	1·44	1·11	9·82	·64	—	15·61	49·91	4·83	1·28
Yellow . . .	·63	1·76	—	—	—	1·14	—	—	1·14	2·84	92·61	—
White	6·57	·46	·46	1·40	·46	1·40	2·81	·46	19·71	55·18	9·38	1·87
Green	—	42·85	—	—	—	—	—	—	—	—	57·14	—

Out of the whole available anthophilous fauna of (for the time of our observations) 17,306 individuals, 1,429 went to Class B. The species obtained attention as in Table XXXIII. In summer among the visitors were more long-tongued Lepidoptera than the class's share, and more Syrphidae (see Table XXXVI); in autumn among the visitors were more Bombi and more Coleoptera because Bombus went very freely to *Thymus* and *Calluna*, and Meligethes to *Raphanus Raphanistrum*. As shown in Table XXXIV, the summer visitors were of a higher type than the spring and autumn visitors.

In Tables XXXVII and XXXVIII the flowers of this class are divided according to their colour, and it is easily seen that the long-tongued Lepidoptera showed a distinct preference for flowers with a certain amount of blue in them, and the Bombi for rose-purple, and Apis for white and rose-purple. One yellow flower, viz. *Raphanus Raphanistrum*, drew so many individuals of Meligethes that the percentages for yellow are biased and the percentages for green are founded on too small figures for discussion of them to be possible.

We do not find Class B, by visitors, breaking into two halves as Class B′ did, yellow and white on one side, blue-lilac-purple and rose on the other, nor do we find the visitors visiting the colours as we found them visiting Classes H and F.

Class B was found to attract nine species of the butterflies, sixteen large moths, six small moths including Eriocephala; of Hymenoptera, Apis, five species of Bombi, two of Andrena, two of Vespa, of Formica, of Odynerus, of Allantus, of four species of Parasitic Hymenoptera; among Diptera, in the Syrphidae of Rhingia campestris, Volucella bombylans, Leucozonia lucorum, Helophilus, three species of Eristalis, one Chrysogaster, seven species of Syrphus, Syritta, Ascia, four species of Platychirus, and two of Melanostoma; in Empidae of seven species of Empis including E. tessellata, of one Rhamphomyia and of one Clinoceira; Siphona geniculata was the only Tachinid attracted; among Muscids the class attracted two Calliphorae, one Lucilia, one Pollenia and one Stomoxys, of Cynomyiidae one species, of Anthomyiids eighteen species including Drymia hamata, of Scatophagids one species, and of other orders of flies eleven species; of Coleoptera six species, one of Hemiptera, one of Trichoptera, and of Thrysanoptera one.

Class Po. B, § 42. Pendant B-like Type.

181. Anemone nemorosa, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 a, 4, 18, 33, 34, Warnstorf 2507.]

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., cp. 18. V. 97; 7–15. V. 96, 800 ft. (2) Bombus lapponicus F., seeking h. and cp. 18–20. V. 97; 15. V. 98, 800 ft. **Diptera.** *Syrphidae*: (3) Platychirus discimanus Lw., fp. 18–20. V. 97; 7. V. 98, 6–800 ft. *Mycetophilidae*: (4) Sciara sp., 16. V. 98,

700 ft. (5) Dilophus sp., 18. V. 97, 800 ft. (6) Scatopse ?, 21. V. 97, 1,600 ft. ? seeking h. 23. V. 96, 2,400 ft. *Muscidae*: (7) Lucilia cornicina F., 20. V. 97, 800 ft. *Anthomyiidae*: (8) Anthomyia sulciventris Ztt., fp. 20–22. V. 97; 7–9. V. 98, 7–1,600 ft. (9) Anthomyia sp., 21. VI. 96; 18–21. V. 97; 15. V. 98; 10. VI. 99, 7–1,500 ft. *Cordyluridae*: (10) Scatophaga stercoraria L., fp. 21. V. 97. 7. V. 98; 8–2,100 ft.

182. Pyrola media, Sw. [Lit. *Alps* 9.] All flowers set seed. Style not decurved as Kerner describes it.

Visitor. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1) 1 sp. 11. VII. 96, 700 ft.

183. Pyrola rotundifolia, Linn. [Lit. *N.C.E.* 1, 4, 14, 24, 25, Warnstorf 2507; *Arct.* 36, 37 a; *Alps* 2.] The style is strongly decurved and the stigma 4–8 mm. from the anthers. All flowers set fruit. Protogyny very slight.

Visitors. **Coleoptera.** (1) Meligethes viridescens F., fp. **Thysanoptera.** (2) Thrips sp. Both 2. VII. 96, 2,000 ft.

TABLE XXXIX.

Individuals visiting the different flowers of Class B-like Po.

Class Po (B)	*Apis.*	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
181. Anemone nemorosa	8	4	—	—	—	—	—	—	—	—	3	202	—	—	217
182. Pyrola media . .	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
183. Pyrola rotundifolia	—	—	—	—	—	—	—	—	—	—	—	—	2	2	4
Total.	8	4	—	—	1	—	—	—	—	—	3	202	2	2	222
Percentage . . .	3·60	1·80	—	—	·45	—	—	—	—	—	1·35	90·99	·90	·90	

Class AB, § 43. Listera Type.

184. Listera cordata, R. Br. [Lit. *Brit.* Darwin 483; *Alps* 2.] The plant has a considerable amount of vegetative reproduction (see J. A. Z. Brundin in Bihang till K. Svenska Vetensk. Akad. Handlingar. XXI. 1875, No. 12). Self-pollination does not occur.

Visitors. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1) 1 sp., 13. VI. 99, 1,500 ft. **Diptera.** *Tipulidae*: (2) Limnophila ? luteolella Verrall, with pollinia adhering to its head and going from flower to flower, sh. 13. VI. 99, 1,800 ft. *Anthomyiidae*: (3) 1 sp., ? seeking h. 19. VI. 96, 1,500 ft. **Coleoptera.** (4) Malthodes sp., three individuals crawling from flower to flower, 27. VI. 96, 1,500 ft.

185. Malaxis paludosa, Sw. [Lit. *Brit.* Darwin 483; *N.C.E.* 14.] This little plant, only 6 cm. high, is very easily overlooked. Its flowers are uniformly green except for the slightly different colours of the lines on

the labellum. All the flowers of one raceme twist so as to face nearly in one direction. The only visitor seen, adhered to the pollinia, but did not pull them out.

Visitors. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1) One sp., seeking h. 5. VII. 96, 800 ft.

Class AB, § 44. Oxalis Type.

186. Oxalis Acetosella, Linn. [Lit. *Brit.* 23. 29; *N.C.E.* 1, 11, 18, 34, 40; *Alps* 2.] In 96 per cent. of the flowers examined the stigmas and stamens were remote from one another, but in 4 per cent. they were near enough to make self-pollination by contact possible.

Visitors. **Lepidoptera.** Heterocera: *Tineidae*: (1) 1 sp., sh. 15. VI. 99, 1,900 ft. **Diptera.** *Empidae*: (2) Empis lucida Ztt., sh. 12–15. VI. 99, 20–2,200 ft. (3) Empis sp., sh. 20. V. 97, 1,200 ft. *Bibionidae*: (4) Scatopse sp., sh. 12. VI. 99, 1,700 ft. *Anthomyiidae*: (5) Hylemyia nigrescens Rnd., sh. 12. VI. 99, 2,500 ft. (6) Anthomyia sp. sh. 23. V. 96; 20–21. V. 97, 12–2,600 ft.

187. Linum catharticum, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 14, 16, Warnstorf 2507; *Pyren.* 17.] The flowers remain closed in bad weather.

Visitors. **Lepidoptera.** Rhopalocera: (1) Coenonympha pamphilus L., 4. VII. 94, about 2,000 ft. **Diptera.** *Tachinidae*: (2) Siphona geniculata Deg., sh. 22. VI. 95, 800 ft. *Anthomyiidae*: (3) 1 sp., 10. VII. 96, 2,200 ft.

Class AB, § 45. Small Ribes Type.

188. Ribes Grossularia, Linn. [Lit. *Brit.* 29, Marquand 1513; *N.C.E.* 1, 3 a, 21 b, 33, 34, 40.] In cultivation and as an escape from it.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., sh. 18–22. V. 97; 7–16. V. 98, 7–800 ft. (2) Bombus terrestris L., sh. 19. V. 97, 8–900 ft. (3) B. lapponicus F., 20. V. 97, 800 ft. *Vespidae*: (4) Vespa ? sylvestris Scop., 20. V. 97, 800 ft. **Diptera.** *Empidae*: (5) Empis lucida Ztt., sh. 13. V. 98, 800 ft. *Bibionidae*: (6) Dilophus sp., sh. and fp. 19–22. V. 97, 800 ft. *Muscidae*: (7) Lucilia cornicina F., 18. V. 97, 800 ft. (8) Pollenia vespillo F., sh. 7. V. 98, 800 ft. *Anthomyiidae*: (9) Anthomyia sulciventris Ztt., fp. 22. V. 97, 800 ft. **Hemiptera.** (10) Anthocoris sp., sh. 19. V. 97, 800 ft.

189. Ribes rubrum, Linn. [Lit. *N.C.E.* 1, 3 a, 16, 40; *Alps* 34.] In cultivation and as an escape from it.

Visitors. **Diptera.** *Mycetophilidae*: (1) Sciara sp., sh. 20. V. 97. *Chironomidae*: (2) Diamesa ? tonsa Hal., sh. 13. V. 98. (3) 1 sp., sh. 20. V. 97. *Muscidae*: (4) Lucilia cornicina F., sh. 18. V. 97. *Anthomyiidae*: (5) Anthomyia sulciventris Ztt., sh. and fp. 24. V. 97; 13. V. 98. **Thysanoptera.** (6) Thrips sp., 18. V. 97. All at 800 ft.

Class AB, § 46. Berberis Type.

190. Berberis Aquifolium, Pursh. [Lit. *N.C.E.* 34.] In cultivation.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus terrestris L., sh. (2) B. lapponicus F., sh. Both 14. V. 98. 800 t.

Class AB, § 47. Acer Type.

191. Acer Pseudo-platanus, Linn. [Lit. *N.C.E.* 3 b, 14, 18, 21 b, 34, Warnstorf 2507.]

Visitors. **Lepidoptera.** Heterocera: *Noctuidae*: (1) 1 sp., 15. VI. 99, 800 ft. **Hymenoptera.** Aculeata: *Apidae*: (2) Apis mellifica L., sh. 21–24. V. 96; 24. V. 97, 7–800 ft. (3) Bombus terrestris L., sh. 21. V. 96; 19–26. V. 97, 7–800 ft. *Vespidae*: (4) Vespa sp., 15. VI. 99, 900 ft. Petiolata parasitica: *Ichneumonidae*: (5) 1 sp. 15. VI. 99, 900 ft. **Diptera.** *Bibionidae*: (6) Bibio nigriventris Hal., 15. VI. 99, 900 ft. *Anthomyiidae*: (7) Trichophthicus sp., sh. 15. VI. 99, 8–900 ft. **Coleoptera.** (8) Meligethes viridescens F., 15. VI. 99, 900 ft. (9) Rhagonica limbata Thoms., 15. VI. 99, 900 ft.

Class AB, § 48. Caltha Type.

192. Caltha palustris, Linn. [Lit. *Brit.* 23, 29, Darwin 485; *N.C.E.* 1, 3 a, 14, 16, 18, 34; *Arct.* 36; *Alps* 2, 21 b; *Pyren.* 17.] Above 1,800 feet we get the variety *minor*; it flowers later than the type. As a result of counting the organs of twenty-eight flowers from 2,600 feet we found the stamens to average nearly 57, and the carpels 4·5 (Cf. Burkill in Journ. Linn. Soc. Bot. xxxi. pp. 233–4).

Visitors. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1 and 2) 2 spp, 22. VI. 95, 2,200 ft. and 15. V. 98, 800 ft. **Diptera.** *Syrphidae*: (3) Chilosia fraterna Mg., sh. 24–26. VI. 95, 8–900 ft. *Empidae*: (4) Empis lucida Ztt., sh. 12. VI. 99, 2,500 ft. (5) E. ? borealis L., 27. V. 97, 800 ft. (6) Rhamphomyia sulcata Fln., ? sh. 10. VI. 99, 700 ft. (7) Clinoceira stagnalis Hal., 19. VI. 95, 2,600 ft. *Bibionidae*: (8) Dilophus albipennis Mg., sh. 22. VI. 95, 2,400 ft. *Chironomidae*: (9) 1 sp., sh. 15. V. 98, 800 ft. *Tachinidae*: (10) Siphona geniculata Deg., 21. V. 96, 800 ft. *Muscidae*: (11) Pollenia vespillo F., 10–12. VI. 99, 700 and 2,500 ft. *Anthomyiidae*: (12) Hyetodesia incana W., 19. VI.–1. VII. 95; 23. V. 96, 8–2,700 ft. (13) Trichophthicus sp., sh. 24. VI. 95, 2,000 ft. (14) Hylemyia nigrescens Rnd., fp. 12. VI. 99, 2,500 ft. ab. (15) Anthomyia sulciventris Ztt., 19. VI. 95, 2,600 ft.; 22. V. 96, 2,400 ft.; 18–27. V. 97, 6–800 ft. (16 and 17) Anthomyia spp. fp. and seeking h. 19–25. VI. 95; 22–23. V. 96; 20. V. 97; 13–16. V. 98; 10–14. VI. 99, 7–2,600 ft. (18) Azelia aterrima Mg., 26. VI. 95, 800 ft. *Phoridae*: (19) Phora rufipes Mg., fp. and seeking h. 22. V. 96, 2,400 ft. **Coleoptera.** (20) Meligethes viridescens F., sh. 22–24. VI. 95, 16–2,200 ft. (21) M. aeneus F., fp. and sh. 21. V. 96; 20. V. 97; 10. VI. 99, 7–900 ft. (22) Anthobium lapponicum Mann., fp. 21. V. 96, 800 ft. **Hemiptera.** (23) Lygus ?, 10. VI. 99, 700 ft. **Neuroptera.**

(24) Chloroperla sp., 1. VII. 95, 800 ft. (25) Nemoura sp., 22. VI. 95, 2,000 ft. **Araneida.** (26) Oligolophus sp. pushing itself amongst the stamens, 12. VI. 99, 700 ft. (27) A spider lying in wait, 20. VI. 97, 8–900 ft. twice.

Class AB, § 49. Oxycoccos Type.

193. **Vaccinium Oxycoccos,** Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 c, 14, 34, 35, Warnstorf 2507; *Arct.* 36, 37 b; *Alps* 2, 9.]

Visitors. **Hymenoptera.** Petiolata parasitica: (1) 1 small sp. **Diptera.** *Bibionidae*: (2) Scatopse sp. Both seeking h. 10. VI. 99, 700 ft.

Class AB, § 50. Small Crucifer Type.

194. **Barbarea vulgaris,** R. Br. [Lit. *Brit.* 23; *N.C.E.* 1, 3 a, 18, 33, 34.]

Visitor. **Coleoptera.** (1) Meligethes viridescens F., fp. and sh. on old and half-withered flowers, 10. VI. 99, 700 ft.

195. **Cochlearia officinalis,** Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 14, 25, 31, 35, Hildebrandt 1051.] There are two types (with intermediates) at Clova. The larger form, which grows in very wet places on the crags, has the anthers and stigma remote from one another by 1 mm. but on the same level: in the closing of the flower, on withering, self-pollination invariably occurs, fertilization resulting. In the smaller form, which is the plant from Little Kilrannoch, figured in the Journal of Botany, xxx, Plate 326, the anthers and stigma are only ½ mm. apart and the flower is smaller—the diameter from tip of petal to tip of petal being 6–8 mm. Self-fertilization invariably occurs in the withering of the flower. It is noteworthy how variable are the flowers of this smaller type, five petals as figured in the Journal of Botany having been seen by us as well as six. Once seven stamens were found, the normally paired ones on one side being three in number.

Visitors. **Lepidoptera.** Heterocera: *Geometridae*: (1) Larentia salicata Hüb., sh. 22. VI. 95, 2,000 ft. **Diptera.** *Mycetophilidae*: (2) Sciara sp., 16. VI. 99, and ? sh. 22. VI. 95, 2,800 ft. *Bibionidae*: (3) Scatopse sp., sh. 16. VI. 99, 2,800 ft. *Anthomyiidae*: (4) Drymia hamata Fln., sh. 1. VII. 96, 2,000 ft. (5) Trichophthicus sp., sh. 2. VII. 96, 2,800 ft. (6 and 7) Anthomyia spp., sh. 22. VI. 95; 16. VI. 99, 25–2,800 ft. **Coleoptera.** (8) Anthophagus alpinus Payk., sh. 1. VII. 96, 2,000 ft. (9) Ceuthorrhynchius contractus Marsh, sh. 1. VII. 96, 2,000 ft. **Neuroptera.** (10) 1 sp., sh. 1. VII. 96, 2,200 ft.

196. **Brassica Sinapis,** Visiani. [Lit. *N.C.E.* 1, 3 b, 8, 11, 14, 18, 31, 32, 33, 34, 40; de Vries 2460.]

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., 22–30. VI. 95.

Diptera. *Empidae*: (2) Empis tessellata F., sh. 30. VI. 95. **Coleoptera.** (3) Meligethes viridescens F., ? sh. 5. VII. 95. All at 800 ft.

197. Capsella Bursa-pastoris, Moench. [Lit. *Brit.* 23, 29, A. Bateson 151; *N.C.E.* 1, 3 b, 11, 14, 18, 25, 31, 33, 34, 40; *Alps* 34; *Pyren.* 17.] Flowers were found to have been—all of them—♀ in the early part of 1897 up to May 20th, when some began to perfect stamens.

Visitors. **Diptera.** *Tachinidae*: (1) Siphona geniculata Deg., sh. 18. IX. 95. *Anthomyiidae*: (2) 1 sp. 21. VI. 96. Both at 800 ft.

198. Iberis amara, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 40; *Alps* 9; *Pyren.* 17.] In cultivation.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris napi L., sh. 11. VI. 99. Heterocera. *Geometridae*: (2) Fidonia atomaria L., sh. 11. VI. 99. **Diptera.** *Syrphidae*: (3) Syrphus vitripennis Mg., fp. and sh. 11–13. VI. 99. freq. (4) Eristalis arbustorum L., sh. 11–12. VI. 99. (5) E. ? pertinax L., sh. 23. V. 97. *Empidae*: (6) Empis opaca F., 12. VI. 99, 800 ft. *Tachinidae*: (7) Siphona geniculata Deg., 11. VI. 99. *Muscidae*: (8) Lucilia sp. 13. VI. 99. *Anthomyiidae*: (9) Hylemyia nigrescens Rnd., 13. VI. 99. (10) Anthomyia radicum L., 11–12. VI. 99. (11) A. sulciventris Ztt., fp. 23. V. 97. **Coleoptera.** (12) Meligethes viridescens F., fp. 10. VI. 99. (13) Psylliodes napi Koch, fp. 10. VI. 99. All at 800 ft.

Class AB, § 51. Trollius Type.

199. Trollius europaeus, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 11, 21 b, 34; *Arct.* 7, Feilden and Geldart in Trans. Norfolk and Norwich Nat. Soc. VI. p. 162; *Alps* 2, 9, 21 b.] *Trollius* is *par excellence* a shelter-flower. It closes in bad weather, and then there are generally many insects harbouring inside. Colonel Feilden used it in Russian Lapland as a means of collecting insects: 'when a sudden change took place, the globe-flower closed its petals into a tight ball but not before the flies, beetles, and many other kinds of insects had fled for refuge to its sheltering bowers'. At Clova self-fertilization may be common; setting of seed is invariable.

Visitors. **Hymenoptera.** Petiolata parasitica. *Proctotrypidae*: (1) 1 sp., 15. VI. 99, 1,900 ft. **Diptera.** *Syrphidae*: (2) Chilosia sparsa Lw., 22. V. 97, 500 ft. *Anthomyiidae*: (3) Hyetodesia incana W., 20. VI. 95, 2,600 ft. (4) Drymia hamata Fln., 20. VI. 95, 2,100 ft. (5) Trichophthicus sp. 20. VI. 96, 2,300 ft. (6) Anthomyia sulciventris Ztt., 22. V. 97; 15. VI. 99, 500 and 1,900 ft. **Coleoptera.** (7) Meligethes aeneus F., 22. VII. 97; 15. VI. 99; 500 and 1,900 ft. (8) Anthophagus alpinus Payk., fp. 22. VI. 96, 2,400 ft. (9) Anthobium torquatum Marsh., 22. VI. 97, 15. VI. 99, 500 and 1,900 ft. All these insects were taken from the interior of flowers, where by reason of the structure of the perianth it is rarely possible to ascertain what they may be doing.

CLASS AB, § 52. PHILADELPHUS TYPE.

200. Philadelphus coronarius, Linn. [Lit. *N.C.E.* 1, 3 b, 18, 32, 34.] In cultivation.

Visitors. **Lepidoptera.** Heterocera: *Noctuidae*: (1) Noctua ?, sh. 2. VII. 96, 800 ft.

CLASS AB, § 53. RHODIOLA TYPE.

201. Sedum Rhodiola, DC. [Lit. *Brit.* 23; *N.C.E.* 21 b; *Arct.* 7, 36, 38; *Alps* 2, 34.] This alpine was found to be visited by flies in Novaya Zembya, and by flies and ants in the Alps.

Visitors. **Hymenoptera.** Petiolata parasitica. *Proctotripidae*: (1) 1 sp., 15. VI. 99, 1,900 ft. **Diptera.** *Syrphidae*: (2) Platychirus manicatus Mg., fp. 23. V. 96, 2,400 ft. *Empidae*: (3) Empis lucida Ztt., 22. VI, 96, 2,400 ft. (4) Rhamphomyia sp., sh. 22. VI. 95, 25–2,600 ft. (5) Hilara canescens Ztt., fp. 23. VI. 96, 2,400 ft. *Bibionidae*: (6) Scatopse sp., 15. VI. 99, 1,900 ft. *Anthomyiidae*: (7) Hyetodesia incana W., 20. VI. 95; 15. VI. 99, 19–2,600 ft. (8) Hylemyia nigrescens Rnd., 15. VI. 99, 1,900 ft. (9) Anthomyia sulciventris Ztt., fp. 23. V. 96, 2,400 ft. (10) Anthomyia sp., ? fp. 22. VI. 96; 17. VI. 99, 2,400 ft. *Psilidae*: (11) Psila fimetaria L., sh. 22. VI. 95, 2,000 ft. **Coleoptera.** (12) Meligethes viridescens F., 20. VI. 96, 2,400 ft. (13) M. aeneus F., sh. 22. VI. 95; 15. VI. 99, 19–2,000 ft.

CLASS AB, § 54. MALUS TYPE.

202. Pyrus intermedia, Ehrh. Planted.

Visitors. **Hymenoptera.** Petiolata parasitica. *Proctotrypidae*: (1) 1 sp., sh. 19. VI. 99. **Diptera.** *Empidae*: (2) Empis tessellata F., sh. 18. VI. 99. (3) Rhamphomyia sulcata Fln., 18–19. VI. 99. *Bibionidae*: (4) Bibio nigriventris Hal., 18. VI. 99. *Muscidae*: (5) Calliphora erythrocephala Mg., sh. 19. VI. 99. *Anthomyiidae*: (6) Anthomyia radicum L., sh. 19. VI. 99. (7) Anthomyiid., 18. VI. 99. **Coleoptera.** (8) Meligethes viridescens F., 18. VI. 99. (9) Phyllobius pyri L., 18. VI. 99. All at 800 ft.

203. Pyrus Malus, Linn. [Lit. *Brit.* 34; *N.C.E.* 1, 40, Plateau, 2002 *N. Am.* Waite 2469.] In cultivation.

Visitors. **Diptera.** *Empidae*: (1) Rhamphomyia sulcata Fln., 12. VI. 99. *Bibionidae*: (2) Bibio nigriventris Hal., seeking h. 10. VI. 99. *Simuliidae*: (3) Simulium sp., sh. 10. VI. 99. *Anthomyiidae*: (4) Anthomyia sp., fp. 10–12. VI. 99. *Sepsidae*: (5) Sepsis sp., 10. VI. 99. **Coleoptera:** (6) Meligethes aeneus F., fp. 10–12. VI. 99, abundant. All at 800 ft.

CLASS AB, § 55. RANUNCULUS TYPE.

204. Ranunculus Auricomus, Linn. [Lit. *Brit.* 23, Winter in Journ. Bot. 1897, p. 406; *N.C.E.* 1, 3 a, 18, 33, 34, Warnstorf 2507; *Arct.* 36.]

One observer—Jungner (in Bot. Notiser, 1894, p. 156)—says that it is slightly anemophilous in Sweden.

Visitor. **Diptera.** *Anthomyiidae*: (1) Anthomyia sulciventris Ztt., 20. V. 97, 800 ft.

205. Ranunculus Flammula, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 a, 14, 18, 21 b, 25, 34.]

Visitors. **Lepidoptera.** Heterocera: *Geometridae*: (1) Larentia salicata Hb., sh. 10. VII. 96, 1,500 ft. *Eriocephalidae*: (2) Eriocephala calthella L., 25. VI. 95, 900 ft. **Diptera.** *Syrphidae*: (3) Chilosia fraterna Mg., sh. 25–26. VI. 95; 18. VI. 96, 800 ft. (4) Chrysogaster hirtella Lw., sh. 1. VII. 95, 800 ft. *Empidae*: (5) Empis tessellata F., 1. VII. 95, 800 ft. *Bibionidae*: (6) Dilophus albipennis Mg., sh. 1. VII. 95, 900 ft. *Anthomyiidae*: (7) Hyetodesia incana W., 26. VI.–2. VII. 95; 18. VI. 96, 800 ft. (8) Spilogaster nigrivenis Ztt., 19. VI. 96, 1,500 ft. (9) Drymia hamata Fln., 21. VI. 95, 900 ft. (10) Hylemyia nigrescens Rnd., sh. 25. VI. 95; 13. VI. 99, 800 ft. (11) Trichophthicus sp., sh. and fp. 25. VI.–6. VII. 95; 16. IX. 95, 8–1,200 ft. (12, 13, and 14) Anthomyia 3 spp., sh. and fp. 21. VI.–6. VII. 95; 13–21. IX. 95; 18. VI.–11. VII. 96, 7–2,300 ft. *Sciomyzidae*: (15) Tetanocera ferruginea Fln., 1. VII. 95, 800 ft. **Coleoptera.** (16) Meligethes viridescens F., sh. and fp. 21–26. VI. 95; 16. IX. 95; 16. VI. 96, 7–900 ft. (17) Donacia discolor Panz., sh. 21. VI.–6. VII. 95, 5–900 ft.

206. Ranunculus bulbosus, Linn. [Lit. *Brit.* 23, 34; *N.C.E.* 1, 3 a, 14, 16, 21 b, 33, 34; *Alps* 2; *Pyren.* 17.]

Visitor. **Diptera.** *Anthomyiidae*: (1) Drymia hamata Fln., 20. V. 96, 1,400 ft.

207. Ranunculus acris, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 a, 11, 14, 14 a, 16, 18, 21 b, 25, 30, 32, 40; *Arct.* 7, 36; *Alps* 2, 16; *Pyren.* 17.] No one has found such great variety of flies on this plant as we have. It is to be remarked that they are allotropous chiefly. This buttercup is very common and of wide range, and its flowers vary considerably in size; we have also found them with contabescent anthers. Müller observed eleven species of Lepidoptera on it in the Alps. The flowers hang in heavy rain, then serving as a shelter to Anthomyiids, e. g. Anthomyia sulciventris.

Visitors. **Lepidoptera.** Rhopalocera: (1) Pieris brassicae L., 15. VI. 99, 800 ft. (2) Lycaena icarus Rott., sh. 26. VI. 95, 800 ft. Heterocera: *Eriocephalidae*: (3) Eriocephala calthella L., sh. 24. VI.–3. VII. 95, 9–1,000 ft. **Hymenoptera.** Aculeata: *Apidae*: (4) Bombus terrestris L., sh. 15. VI. 99, 900 ft. (5) Andrena coitana Kirby, 5. VII. 95, 800 ft. Sessiliventres: *Tenthredinidae*: (6) Allantus arcuatus Forst., devouring the flower, 14. VI.–21. VII. 95; 29. VI.–6. VII. 96; 19. VI. 99, very freq. Petiolata parasitica: *Ichneumonidae*: (7) 1 sp., sh. 4. VII. 95; 11. VII. 96, 800 ft. *Proctotrypidae*: (8) Proctotrype ? sh. 21. IX. 95, 800 ft. *Chalcididae*: (9 and 10) 2 spp., 27. VI.–6. VII. 95, 8–1,000 ft. (also 11) 1 sp., 26. VI. 95, 21–2,200 ft. **Diptera.** *Syrphidae*: (12) Chrysogaster hirtella Lw., sh. 8.

VII. 95, 800 ft. (13) Chilosia fraterna Mg., sh. 22–24. VI. 95; 18–29. VI. 96; 15–16. VI. 99, 8–1,500 ft. (14) Platychirus manicatus Mg., sh. 17. VII. 95; 29. VI. 96; 10. VI. 99, 7–1,500 ft. (15) P. albimanus F., sh. and fp. 22–24. IX. 95, 8–1,000 ft. (16) P. peltatus Mg., sh. 21. VI.–3. VII. 95, 900 ft. (17) P. clypeatus Mg., 29. VI.–2. VII. 95, 8–1,000 ft. (18) Melanostoma mellinum L., sh. and fp. 1. VII. 95, 13–21. IX. 95, 7–800 ft. (19) Syrphus luniger Mg., 15–16. VI. 99, 800 ft. (20) S. ? ribesii L. 16. VI.–3. VII. 96. 7–800 ft. (21) Eristalis arbustorum L., sh. 22. VI. 95, 800 ft. *Empidae*: (22) Empis punctata Mg., sh. 15–20. VII. 95, 800 ft. (23) E. aestiva Lw., sh. 21. IX. 95, 800 ft. (24) Rhamphomyia spinipes Fln., ? sh. 16. IX. 95, 800 ft. (25) R. albosegmentata Ztt., sh. 22. VI. 95, 1,400 ft. (26) Hilara matrona Hal., sh. 17. VII. 95, 800 ft. *Mycetophilidae*: (27) Sciara sp., 20. VI. 95, 1,700 ft. *Bibionidae*: (28) Dilophus albipennis Mg., sh. and fp. 15–16. VI. 95, 900 ft., and 25. VI. 96, 2,200 ft. *Tachinidae*: (29) Siphona geniculata Deg., sh. 4. VII. 95; 17. IX. 95, 800 ft. *Sarcophagidae*: (30) Sarcophaga sp., 10. VII. 96. 800 ft. *Muscidae*: (31) Lucilia cornicina F., sh. 24. IX. 95, 1,600 ft. (32) Pollenia rudis F., fp. 22. IX. 95, 800 ft. *Anthomyiidae*: (33) Hyetodesia incana W., sh. 17. VI.–15. VII. 95; 16. IX. 95; 18. VI.–11. VII. 96, 8–2,500 ft. ab. (34) H. lucorum Fln., 25. VI. 95, 1,000 ft. (35) H. basalis Ztt., sh. 20. VII. 95, 800 ft. (36) H. sp., 15–25. VI. 95, 8–1,400 ft. (37) Spilogaster nigrivenis Ztt., 27. VI. 96, 1,400 ft. (38) Limnophora solitaria Ztt., 28. VI. 95, 2,200 ft. (39) Limnophora sp., sh. 1. VII. 95; 19. VI. 96, 11–1,200 ft. (40) Drymia hamata Fln., sh. and fp. 20. VI.–10. VII. 95; 16. IX. 95; 20. VI.–1. VII. 96, 8–2,400 ft. (41) Trichophthicus hirsutulus Ztt., sh. 6. VII. 96, 2,000 ft. (42) Trichophthicus sp., sh. 16. VI.–15. VII. 95; 20. VI.–1. VII. 96, 8–2,400 ft. (43) Hylemyia variata Fln., sh. 21. VI. 95, 900 ft. (44) H. nigrescens Rnd., 13. VI. 99, 800 ft. (45) Anthomyia sulciventris Ztt., sh. 28. VI. 95; 27. V. 97, 700 and 2,200 ft. (46) A. radicum L., fp. 11–16. VI. 99, 8–2,000 ft. (47 and 48) Anthomyia spp., sh. and fp. 15. VI.–22. VII. 95; 13–24. IX. 95; 16. VI.–10. VII. 96; 10–19. VI. 99, 7–2,600 ft. (49) Homalomyia sp., sheltering 18. VI. 95, 900 ft. (50) Azelia aterrima Mg., 29. VI. 96, 900 ft. (51) Coenosia infantula Rnd., sh. 21. VI.–6. VII. 95, 8–1,700 ft. *Cordyluridae*: (52) Scatophaga stercoraria L., 19. VI.–4. VII. 95; 14–24. IX. 95, 8–2,700 ft. (53) S. maculipes Ztt., sh. 21. VI. 95, 900 ft. (54) S. squalida Mg., 4. VII. 95, 2,300 ft. *Psilidae*: (55) Psila sp., sh. 12. VII. 95, 700 ft. *Sepsidae*: (56) Sepsis cynipsea L. sh. 19. VI. 95; 25. VI. 96, 800 ft. *Ephydridae*: (57) Hydrellia griseola Fln., 4. VII. 95, 800 ft. *Chloropidae*: (58) Oscinis sp. 4. VII. 95; 26. VI. 96, 800 and 2,100 ft. *Agromyzidae*: (59) Agromyza sp., sh. 19. VI. 95, 800 ft. **Coleoptera.** (60) Meligethes viridescens F., sh. and fp. 19. VI.–4. VII. 95; 17–24. IX. 95; 16–27. VI. 96; 19. VI. 99, 7–2,700 ft. ab. (61) M. aeneus F., sh. 21. VI.–6. VII. 95; 19. VI. 99, 8–1,600 ft. (62) Anthophagus alpinus Payk., sh. 4. VII. 95; 27. VI.–2. VII. 96, 19–2,600 ft. (63) Anthobium minutum F., 24. VI. 95, 1,500 ft. (64) A. lapponicum Mann., 1. VII. 96, 1,600 ft. (65) Tachyporus ? hypnorum F., 19. VI. 99, 800 ft. (66) Chrysomela staphylea L., devouring the flowers, 3. VII. 96, 2,500 ft. (67) Dascillus cervinus L., 1–4. VII. 95, 800 ft. **Hemiptera.** (68) Acocephalus sp. 25. VI. 95, 1,700 ft. (69) Leptopterna dolabrata L., 4 VII. 95, 800 ft. **Thysanoptera.** (70) Thrips sp., 15. IX. 95, 800 ft.

208. Ranunculus repens, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 a, 14, 16, 21 b, 25, 31, 34, 40; *Arct.* 7, 36; *Alps* 16, 21 a.] Freely visited by Anthomyiids.

Visitors. **Hymenoptera.** Sessiliventres: *Tenthredinidae*: (1) Allantus arcuatus Forst., lounging in flowers, 19. VI. 95, 800 ft. **Diptera.** *Syrphidae*: (2) 1 sp. 14. VI. 95, 700 ft. *Tachinidae*: (3) Siphona cristata F., 22. VI. 95, 800 ft. *Anthomyiidae*: (4) Hyetodesia incana W., sh. 17–22. VI. 95; 18. VI.–4. VII. 96, 800 ft. (5) Trichophthicus sp., fp. 20. VI. 96, 2,300 ft. (6) Anthomyia sulciventris Ztt., sh. and fp. 15. VI. 95; 27. V. 97, 7–800 ft. (7) Anthomyia sp., 22. VI. 95, 800 ft. *Sepsidae*: (8) Sepsis cynipsea L., sh. 19. VI. 95, 800 ft. **Coleoptera.** (9) Meligethes viridescens F., sh. 29. VI. 96, 800 ft.

209. Ranunculus Ficaria, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 a, 3 b, 3 c, 4, 8, 9, 10, 11, 12, 18, 21 b, 33, 34, 40, Warnstorf 2507, Hennings in Verhandl. bot. Ver. Brandenburg xxxvii., p. xxii, Delpino in Bull. Orto bot. Univ. Napoli, i. 1899, p. 24.]

Visitors. **Diptera.** *Muscidae*: (1) Lucilia cornicina F., sh. 7. V. 98, 800 ft. *Anthomyiidae*: (2) Anthomyia sulciventris Ztt., 22. V. 97, 800 ft. (3) Anthomyia sp., sh. and fp. 20–22. V. 97; 16. V. 98, 6–900 ft. **Araneida.** (4) Xysticus sp., lying in wait, 20. V. 97, 900 ft.

Class AB, § 56. Potentilla Type.

210. Fragaria vesca, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 18, 21 b, 33, 34, 40; *Alps* 2; *Pyren* 17.]

Visitor. **Diptera.** *Anthomyiidae*: 1 sp., 2. VII. 96, 2,100 ft.

211. Potentilla Fragariastrum, Ehrh. [Lit. *Brit.* 29; *N.C.E.* 18; *Pyren.* 17.]

Visitors. **Hymenoptera.** Petiolata parasitica. (1) 1 sp. **Diptera.** *Chironomidae*: (2) 1 sp. *Anthomyiidae*: (3) Anthomyia sp., sh. fairly freq. All. 14. V. 98, 1,400 ft.

212. Potentilla Tormentilla, Neck. [Lit. *Brit.* 23, 39; *N.C.E.* 1, 3 b, 4, 14 a, 18, 21 a, 40; *Alps* 2; *Pyren.* 17.] The very abundance of this secured for it a great deal of observation and a consequently long list of visitors.

Visitors. **Lepidoptera.** Rhopalocera: (1) Coenonympha pamphilus L., sh. 15–20. VI. 95; 1. VII. 96, 9–1,400 ft. (2) Lycaena icarus Rott., sh. 25. VI. 95, 800 ft. Heterocera: *Tineidae*: (3) Glyphypteryx fuscoviridella Haw., 20. VI. 95, 1,300 ft. *Eriocephalidae*: (4) Eriocephala calthella L., sh. and fp. 2–21. VII. 95, 8–1,700 ft. **Hymenoptera.** Aculeata: *Apidae*: (5) Bombus terrestris L., sh. 14. IX. 95, 800 ft. (6) Andrena analis Panz., sh. 6. VII. 95; 22. VI.–6. VII. 96, 9–1,500 ft. (7) A. coitana Kirby, sh. 6. VII. 95, 800 ft. *Formicidae*: (8) Formica fusca Latr., sh. 25. VI.–17. VII. 95; 19–26. VI. 96; 15–17. VI. 99, 8–2,100 ft.

Myrmicidae: (9) Myrmica rubra L., sh. 16–23. VI. 95; 19. VI. 99, 8–900 ft. Petiolata parasitica: *Ichneumonidae*: (10) 1 sp., sh. 22. V.–3. VI. 95; 23. V. 97, 10–1,300 ft. *Chalcididae*: (11 and 12) 2 spp., 15–29. VI. 95; 29. VI. 96; 11. VI. 99, 8–2,500 ft. **Diptera.** *Syrphidae*: (13) Platychirus manicatus Mg., 21. VII. 95; 25. VI. 96, 7–1,000 ft. (14) P. clypeatus Mg., 29. VI. 95, 1,000 ft. (15) P. albimanus F., 21. IX. 95, 800 ft. (16) Melanostoma mellinum L., 20. VI. 95, 1,000 ft. (17) Chilosia fraterna Mg., sh. 26. VI. 95; 19. VI. 96, 8–1,300 ft. (18) Chrysogaster hirtella Lw., sh. 22. VI. 95, 800 ft. (19) Syrphus sp., 20. VI. 95, 900 ft. (20) Ascia ? podagrica F., 26. VI.–1. VII. 95, 800 ft. (21) Sphaerophoria scripta L., sh. 19. VI. 96, 2,200 ft. (22) S. nitidicollis Ztt., 20. VI. 95, 1,600 ft. *Empidae*: (23) Empis lucida Ztt., 12–14. VI. 99, 14–1,600 ft. *Bibionidae*: (24) Scatopse sp., 29. VI. 96, 1,500 ft. *Dolichopodidae*: (25) Dolichopus atratus Mg., 22. VI. 96, 800 ft. (26) D. rupestris Hal., sh. 6. VII. 95, 1,500 ft. (27) Hercostomus nigripennis Fln., 1. VII. 95, 800 ft. *Tachinidae:* (28) Siphona geniculata Deg., 18. VI. 99, 800 ft. *Muscidae*: (29) Calliphora erythrocephala Mg., 4. VII. 95; 16. VI. 99, 8–1,500 ft. *Anthomyiidae*: (30) Hyetodesia incana W., 26–29. VI. 95, 1,000 and 2,400 ft. (31) Spilogaster nigrivenis Ztt., 30. VI. 96, 2,100 ft. (32) Limnophora sp., sh. 19. VI. 96, 15–1,600 ft. (33) Drymia hamata F., 20. VI.–6. VII. 95; 20. VI.–1. VII. 96, 8–2,500 ft. (34) Trichophthicus hirsutulus Ztt., 20. VI. 95; 6. VII. 96, 16–2,000 ft. (35) Hylemyia nigrescens Rnd., sh. 10–11. VI. 99, 7–1,300 ft. (36) Anthomyia radicum L., 10–18. VI. 99, 7–2,000 ft. (37) A. sulciventris Ztt., 20. VI. 95; 20–27. V. 97, 5–2,500 ft. (38 and 39) Anthomyia 2 spp., sh. and fp. 20. VI. 95; 14–21. IX. 95; 22. V. 96; 19–22. VI. 96, 8–2,500 ft. (40) Coenosia sp., fp. 19–20. VI. 95; 27. VI. 96; 11. VI. 99, 8–2,000 ft. *Cordyluridae*: (41) Scatophaga sp., sh. 19. VI. 99, 2,200 ft. *Ortalidae*: (42) Pteropaectria frondescentiae L., sh. 1–6. VII. 95, 9–1,700 ft. *Ephydridae*: (43) 1 sp. 15. VI. 95, 900 ft. *Chloropidae*: (44) Oscinis sp., 20. VI 95, 8–1,200 ft. *Phytomyzidae*: (45) Phytomyza sp., 14. IX. 95, 1,300 ft. *Phoridae*: (46) Phora sp., 20. VI. 95; 20. IX. 95, 10–1,300 ft. **Coleoptera.** (47) Meligethes viridescens F., sh. and fp. 24–29. VI. 95; 21. IX. 95; 19–25. VI. 96, 7–2,500 ft. (48) M. aeneus F., 1. VII. 95, 1,500 ft. **Hemiptera.** (49) 1 sp., 10. VI. 99, 700 ft.

213. Potentilla maculata, Pourr. (*P. aurea*, Linn.). [Lit. *Brit.* 23; *Alps* 2, 21 a, 21 b; *Pyren.* 17.] The flower is 18–20 mm. across and very conspicuous. The mechanism seems to agree closely with that of *P. Tormentilla*. Honey is present in fair quantity.

Visitors. **Diptera.** *Syrphidae*: (1) Platychirus sp., 17. VI. 99, 2,300 ft. *Anthomyiidae*: (2) Drymia hamata Fln., seeking h. 20. VI. 96, 2,300 ft. (3) Anthomyia sulciventris Ztt., 25. VI. 96, 2,200 ft. **Araneida.** (4) Xysticus sp., lying in wait, 17. VI. 99, 2,300 ft.

214. Potentilla Comarum, Nestl. [Lit. *Brit.* 23; *N.C.E.* 1, 8, 12, 14, 14 a, 18, 88; *Arct.* 84.] At the opening of the bud the stamens standing erect dehisce; then they move outwards and the anthers fall off. But it would seem that the outermost stigmas are receptive before all chance of pollination from the innermost stamens has passed away. The central

stigmas are then left to be fertilized by some outside agent. The stamens are of three lengths, respectively 4, 5, and 6 mm., the shortest are the innermost before the petals, the longest those before the sepals. Honey is abundant and protected by hairs.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Andrena ? analis Panz., sh. 19. VI. 96. Petiolata parasitica. (2) 1 sp., sh. 27. VI. 96. **Diptera.** *Empidae*: (3) Empis bilineata Lw., 4. VII. 95. (4) E. stercorea L., sh. 1. VII. 95. *Chironomidae*: (5) Ceratopogon sp., sh. 18–27. VI. 96. *Tipulidae*: (6) Limnophila lineolella Verrall, sh. 5. VI. 95; 18. VI. 96. *Anthomyiidae*: (7) Hyetodesia incana W., sh. 28. VI.–1. VII. 95; 18. VI.–4. VII. 96, fairly freq. **Coleoptera.** (8) Meligethes viridescens F., sh. 18. VI. 96. (9) M. aeneus F., sh. 1. VII. 95. All at 800 ft.

Class AB, § 57. Parnassia Type.

215. Parnassia palustris, Linn. [Lit. *Brit.* 23, 89; *N.C.E.* 1, 11, 14, 24, 25, 34, 35, 40; *Arct.* 36; *Alps* 2, 9; *Pyren.* 17.]

Visitors. **Hymenoptera.** Aculeata: *Formicidae*: (1) Formica fusca Latr., seeking h. 16. IX. 95, 12–1,300 ft. *Myrmicidae*: (2) Myrmica rubra L., sh. and seeking h. 16. IX. 95, 12–1,300 ft. Petiolata parasitica. *Ichneumonidae*: (3) 1 sp., sh. 16. IX. 95, 1,300 ft. *Braconidae*: (4) 1 sp., 16. IX. 95, 1,300 ft. **Diptera.** *Cecidomyiidae*: (5) Cecidomyia sp., sh. 16. IX. 95, 1,200 ft. *Anthomyiidae*: (6) Hyetodesia incana W., fp. 16. IX. 95, 1,200 ft. (7) Trichophthicus sp., 16. IX. 95, 1,200 ft. (8) Anthomyia sp., 13. IX. 95, 700 ft. *Cordyluridae*: (9) Scatophaga stercorea L., sh. 16. IX. 95, 1,300 ft. **Coleoptera.** (10) Meligethes viridescens F., sh. 16. IX. 95, 12–1,300 ft. (11) Epuraea aestiva L., sh. 16. IX. 95, 1,200 ft. **Thysanoptera.** (12) Thrips sp., sh. 13–21. IX. 95, 7–1,000 ft.

Class AB, § 58. Intermediate Saxifraga Type.

216. Saxifraga nivalis, Linn. [Lit. *Arct.* 36, 37 b.] It seems to be abundantly self-fertilized.

Visitors. **Hymenoptera.** Petiolata parasitica. (1) 1 sp. 10. VII. 96, 2,000 ft.

217. Saxifraga hypnoides, Linn. [Lit. *Brit.* 23.] It is fairly well visited by allotropous insects.

Visitors. **Lepidoptera.** Heterocera: *Geometridae*: (1) Psodos trepidaria Tr., sh. 22. VI. 95, 800 ft. *Tortricidae.* (2) 1 sp. 22. VI. 96, 2,500 ft. **Hymenoptera.** Petiolata parasitica. *Ichneumonidae*: (3, 4, and 5) 3 sp., sh. 17–24. VI. 95; 26. VI.–6. VII. 96, 16–2,500 ft. *Cynipidae*: (6) 1 sp., sh. 22. VI.–4. VII. 95, 18–27,000 ft. Sessiliventres. *Tenthredinidae*: (7) Nematus carinatus Htg., sh. 17. VI. 99, 2,400 ft. **Diptera.** *Syrphidae*: (8) Platychirus manicatus Mg., sh. 22. VI. 95, 1,800 ft. *Empidae*: (9) Empis tessellata F., sh. 22. VI. 95, 2,000 ft. (10) E. lucida Ztt., 22. VI. 96, 23–2,400 ft. (11) E. aestiva Lw., sh. 30. VI. 96, 2,100 ft. (12 and 13) Empis spp., 22. VI. 95, 2? ♀. *Cecidomyiidae*: (14) Sciara sp.,

30. VI. 96, 2,100 ft. *Bibionidae*: (15) Dilophus albipennis Mg., sh. 22. VI. 95, 18–2,300 ft. *Chironomidae*: (16) Tanypus nebulosus Mg., sh. 15. VII. 95, 2,400 ft. (17) Another Chironomid, sh. 17. VI. 95, 1,800 ft. *Limnobidae*: (18) Dicranomyia morio F., sh. 22. VI.–3. VII. 96, 22–2,400 ft. *Anthomyiidae*: (19) Drymia hamata Fln., sh. 22. VI. 95; 1. VII. 96, 18–2,400 ft. (20) Trichophthicus hirsutulus Ztt., sh. 6–20. VII. 96, 23–2,400 ft. (21) Trichophthicus sp., sh. 20. VII. 96, 23–2,400 ft. (22) Anthomyia sulciventris Ztt., 22. VI. 95, 1,800 ft. (23, 24, and 25) Anthomyia 3 spp., sh. 21. VI.–11. VII. 95; 22. VI.–6. VII. 96, 15–2,600 ft. *Cordyluridae*: (26) Scatophaga stercoraria L., fp. 22. VI.–4. VII. 95; 30. VI. 96, 21–2,500 ft. *Phoridae*: (27) Phora rufipes Mg., sh. 3. VII. 96, 2,200 ft. **Coleoptera.** (28) Meligethes viridescens F., sh. 22. VI. 95; 3–6. VII. 96, 18–2,400 ft. (29) Anthophagus alpinus Payk., sh. freq. 3–4. VII. 95; 22. VI.–6. VII. 96, 21–2,600 ft. (30) Helodes marginata F., 24. VI. 95, 1,600 ft. (31) Epuraea aestiva L., sh. 3. VII. 96, 2,400 ft. **Thysanoptera.** (32) Thrips sp., sh. 3–4. VII. 95, 2,600 ft. **Araneida.** (33) Oligolophus sp., once in the flower, 20. VI. 96, 2,100 ft.

Class AB, § 59. Alsine Type.

218. Cerastium triviale, Link. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 a, 3 b, 14, 18, 21 a, 21 b, 25, 34.] Varieties from high up have larger flowers than occur low down. The stigmas of these ultimately recurve so far as to bring about self-pollination. The long stamens dehisce just before the short ones.[1]

Visitors. **Hymenoptera.** Petiolata parasitica. (1) sp., sh. 2. VII. 95, 800 ft. **Diptera.** *Syrphidae*: (2) Platychirus manicatus Mg., sh. and fp. 6. VII. 95; 25. VI. 96, 7–800 ft. *Mycetophilidae*: (3) Sciara ?, sh. 2. VII. 96, 2,800 ft. *Chironomidae*: (4) Orthocladius sp., sh. 29. VI. 95, 1,600 ft. *Anthomyiidae*: (5) Limnophora sp., sh. 1. VII. 95, 800 ft. (6) Anthomyia radicum L., 10–11. VI. 99, 7–800 ft. (7) Trichophthicus sp., 2. VII. 96, 2,800 ft. *Phoridae*: (8) Phora sp., 13. VI. 99, 900 ft. **Thysanoptera.** (9) Thrips sp., sh. 3. VII. 95; 29. VI. 96, 8–1,000 ft.

219. Cerastium alpinum, Linn. [Lit. *Brit.* 23; *Arct.* 7, 34, 36, 37 a, 38; *Alps* 2, 9, 21 b, Ludwig 1394.] The flower is conspicuous, the petals attaining a length of 12 mm. Fruit is set in plenty in July and later, but flowering continues beyond this.

Visitors. **Hymenoptera.** Petiolata parasitica. *Chalcididae*: (1) 1 sp., sh. 17. VI. 99, 2,300 ft. **Diptera.** *Anthomyiidae*: (2) Drymia hamata Fln., sh. 17. VI. 99, 2,300 ft. (3) Trichophthicus sp., sh. and fp. 20. VI. 96, 23–2,400 ft. **Coleoptera.** (4) Meligethes aeneus F., sh. 17. VI. 99, 2,300 ft. **Thysanoptera.** (5) Thrips sp., 17. VI. 99, 2,300 ft.

220. Stellaria media, Cyr. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 b, 14, 18, 21 a, 21 b, 25, 33, Warnstorf 2507; *Alps* 2; *N. Am.* 12 b.] Always self-fertilized in the absence of insect visitors.

Visitors. **Hymenoptera.** Petiolata parasitica. *Ichneumonidae*: (1) 1 sp., 17.

[1] In August, 1905, on the Yorkshire moors north of Scarborough, about 40 per cent. of the flowers of *Cerastium triviale* then open had infertile stamens.

IX. 95, 800 ft. *Braconidae*: (2) 1 sp., sh. 24. IX. 95, 1,000 ft. (3) a second sp., 10. VI. 99, 700 ft. **Diptera.** *Syrphidae*: (4) Platychirus manicatus Mg., sh. 10. VI. 99, 700 ft. *Muscidae*: (5) Calliphora erythrocephala Mg., sh. 17. IX. 95, 800 ft. (6) Pollenia rudis F., sh. 22. IX. 95, 800 ft. *Anthomyiidae*: (7) Anthomyia radicum L., sh. 10. VI. 99, 700 ft. (8) Anthomyia sp., 17–21. IX. 95, 800 ft. **Coleoptera.** (9) Meligethes viridescens F., sh. and fp. 16–22. IX. 95, 800 ft. **Thysanoptera.** (10) Thrips sp., sh. 17–22. IX. 95, 800 ft.

221. Stellaria Holostea, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 b, 18, 21 a, 21 b, 33, 34, 40; *Alps* 2; *Pyren.* 17.]

Visitor. **Diptera.** *Anthomyiidae*: (1) sp. fp. 10. VI. 99, 600 ft.

222. Stellaria graminea, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 14, 18, 21 a, 21 b, 25, 33, 34; *Alps* 2.]

Visitors. **Diptera.** *Syrphidae*: (1) Platychirus manicatus Mg., 5. VII. 95, 800 ft. *Anthomyiidae*: (2) Trichophthicus sp., fp. 16. IX. 95, 900 ft.

223. Sagina procumbens, Linn. [Lit. *Brit.* 23; *N.C.E.* 14, 18, 21 a, 21 b, 34; *Arct.* 37 a.] Self-pollinating.

Visitor. **Diptera.** *Chloropidae*: (1) Oscinis ?, 22. VI. 95, 800 ft.

224. Spergula arvensis, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 9, 14, 14 a, 18, 21 a, 21 b, 24, 33.] Schulz (in Ber. d. deutschen Bot. Gesellsch. xxi, 1903, 119) points out how this plant is self-fertilized.

Visitors. **Diptera.** *Syrphidae*: (1) Platychirus manicatus Mg., 5. VII. 95. (2) P. albimanus F., sh. 22. IX. 95. (3) Syritta pipiens L., 30. VI. 95. *Tachinidae*: (4) Siphona geniculata Deg., sh. 22. IX. 95. *Anthomyiidae*: (5) Trichophthicus sp., sh. 30. VI. 95. (6) Anthomyia sp., sh. 22. IX. 95. **Thysanoptera.** (7) Thrips sp., 30 VI. 95; 22. IX. 95. All at 800 ft.

Class AB, § 60. Loiseleuria Type.

225. Loiseleuria procumbens, Desv. [Lit. *Arct.* 34, 36, 37 a, 38; *Alps* 2, 9.] The flower is slightly protogynous with ultimate self-fertilization on the fall of the corolla and sometimes before. The flowers are very little visited; Bombi pass them over in search of those of *Vaccinium*; they fill with water in bad weather. Yet each one sets seed. Like several other alpines which grow at Clova they are extremely variable, frequently being 6- or 7-merous.

Visitors. **Diptera.** *Mycetophilidae*: (1) Sciara sp., four individuals sh. *Bibionidae*: (2) Scatopse sp. *Anthomyiidae*: (3) 1 sp. All 11. VI. 99, 2,400 ft.

Class AB (? Class Ne), § 61. Salix Type.

226. Salix Caprea, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 b, 16, 33, 34, 40.]

Visitors to male catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lap-

ponicus F., sh. 9–14. V. 98, 8–1,900 ft. (2) B. terrestris L., 9–11. V. 98, 800 ft. **Diptera.** *Syrphidae*: (3) Platychirus discimanus Lw., fp. and sh. 21. V. 97; 14. V. 98, 9–1,300 ft. (4) Eristalis arbustorum L., sh. 14. V. 98, 900 ft. *Empidae*: (5) Empis borealis L., sh. 21. V. 97, 1,300 ft. (6) E. niveipennis sh. 21. V. 97, 1,300 ft. *Muscidae*: (7) Lucilia cornicina F., 21. V. 97, 1,300 ft. *Anthomyiidae*: (8) Anthomyia sulciventris Ztt., sh. and fp. 21. V. 97, 1,300 ft. (9) Anthomyia sp., 9–14. V. 98, 9–1,500 ft. *Cordyluridae*: (10) Scatophaga stercoraria L., sh. 9. V. 98, 800 ft. *Sepsidae*: (11) Sepsis cynipsea L., ? sh. 21. V. 97, 1,300 ft. **Hemiptera.** (12) Anthocoris sp., sh. 14. V. 98, 900 ft.

Visitors to female catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lapponicus F., sh. 9–16. V. 98, 8–1,400 ft. **Diptera.** *Empidae*: (2) Empis lucida Ztt., 16. V. 98, 800 ft. *Chironomidae*: (3) 1 sp., 16. V. 98, 800 ft. *Anthomyiidae*: (4) Anthomyia sp., 14. V. 98. 1,400 ft. all sh.

227. Salix aurita, Linn. [Lit. *Brit.* 23, 29; *N.C.E.* 1, 3 b, 16, 33.] This is well visited, especially its male catkins.

Visitors to male catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., cp. and sh. freq., 19–27. V. 97; 7–16. V. 98, 6–900 ft. (2) Bombus hortorum L., 22. V. 97, 700 ft. (3) B. lapponicus F., sh. 20–22. V. 97; 16. V. 98, 6–900 ft. (4) B. terrestris L., sh. 20–22. V. 97, 6–800 ft. *Vespidae*: (5) Vespa sp., 20. V. 97, 900 ft. *Formicidae*: (6) Formica fusca Latr., sh. 20. V. 97, 800 ft. **Diptera.** *Syrphidae*: (7) Melanostoma quadrimaculatum Verrall, 7. V. 98, 600 ft. (8) Chilosia bergenstammi Becker, sh. 22. V. 97, 600 ft. (8) Platychirus discimanus Lw., sh. and fp. 19–20. V. 97; 7–16. V. 98, 8–900 ft. (9) Sericomyia lappona L., sh. 20. V. 97, 1,200 ft. (10) Eristalis arbustorum L., sh. 16. V. 98, 700 ft. *Empidae*: (11) Empis borealis L., 20–27. V. 97, 6–1,200 ft. (12) Rhamphomyia cinerascens Mg., sh. 20. V. 97, 9–1,200 ft. (13) R. sulcata Fln., sh. 21. V. 96, 700 ft. *Mycetophilidae*: (14) Sciara sp., 16. V. 98, 600 ft. *Bibionidae*: (15) Scatopse sp., sh. 19. V. 97, 800 ft. *Chironomidae*: (16) 1 sp., 27. V. 97, 700 ft. *Muscidae*: (17) Lucilia cornicina F., sh. 19–20. V. 97, 8–1,200 ft. (18) Pollenia vespillo F., sh. 20–27. V. 97; 16. V. 98, 7–1,200 ft. *Anthomyiidae*: (19) Anthomyia sulciventris Ztt., fp. and sh. 20–27. V. 97; 7. V. 98, 7–900 ft. (20) Anthomyia sp., sh. 19. V. 97, 800 ft. (21) Hylemyia ?, sh. 22. V. 97, 700 ft. *Cordyluridae*: (22) Scatophaga stercoraria L., 19. V. 97; 16. V. 98, 7–800 ft.

Visitors to female catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus terrestris L., sh. 22. V. 97; 7. V. 98, 600 ft. (2) B. lapponicus F., 22. V. 97; 7–16. V. 98, 6–800 ft. (3) Andrena clarkella Kirby, sh. 22. V. 97, 600 ft. **Diptera.** *Syrphidae*: (4) Platychirus discimanus Lw. 19. V. 97; 7. V. 98, 6–1,300 ft. (5) Chilosia sparsa Lw., sh. 22. V. 97, 600 ft. *Mycetophilidae*: (6) Sciara sp., 16. V. 98, 600 ft. *Muscidae*: (7) Lucilia cornicina F., 20. V. 97, 900 ft. (8) Pollenia vespillo F., sh. 20. V. 97; 7. V. 98, 6–1,200 ft. *Anthomyiidae*: (9) 1 sp., 19–20. V. 97, 8–1,800 ft. *Cordyluridae*: (10) Scatophaga stercoraria L., sh. 20–22. V. 97; 7–16. V. 98, 6–1,200 ft. **Coleoptera.** (11) Phyllodecta vulgatissima L., sh. 22. V. 97, 700 ft.

228. Salix Lapponum, Linn. [Lit. *Arct.* 34.]

Visitors to male catkins. **Hymenoptera.** Aculeata: *Apidae:* (1) Bombus lapponicus F., sh. 24. V. 97, 2,800 ft. *Vespidae*: (2) Vespa norvegica F., 11. VI. 99, 2,100 ft. **Diptera.** *Mycetophilidae*: (3) Sciara sp., 11. VI. 99, 2,100 ft. *Chironomidae*: (4) 1 sp., 24. V. 97, 2,300 ft. *Anthomyiidae*: (5) Coenosia sp., fp. 24. V. 97; 11. VI. 99, 21–2,300 ft.

Visitor to female catkins. **Diptera.** *Bibionidae*: (1) Scatopse sp., 11. VI. 99, 2,100 ft.

229. Salix repens. Linn. [Lit. *N.C.E.* 1, 14, 25, 33, 34.]

Visitors to male catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., sh. 7. V. 98, 600 ft. (2) Bombus lapponicus F., sh. 22. V. 97, 700 ft. (3) B. terrestris L., 7. V. 98, 600 ft. Sessiliventres. *Tenthredinidae*: (4) Nematus fallax Lep., sh. 18. V. 97, 800 ft. **Diptera.** *Syrphidae.* (5) Platychirus discimanus Lw., sh. and fp. freq. 21–27. V. 97; 7. V. 98, 6–1,400 ft. (6) Syrphus punctulatus Verrall, sh. 22. V. 97, 700 ft. *Empidae*: (7) Empis borealis L., 20–22. V. 97, 7–1,400 ft. (8) E. lucida Ztt., sh. 7. V. 98, 600 ft. (9 and 10) Empis spp., sh. and fp., 21. V. 97, 1,400 ft. *Muscidae*: (11) Lucilia cornicina F., sh. 18. V. 97, 800 ft. (12) Pollenia vespillo F., sh. 7. V. 98, 6–700 ft. *Anthomyiidae*: (13) Anthomyia sulciventris Ztt., sh. and fp. 18–22. V. 97; 16. V. 98, 6–900 ft. (14 and 15) Anthomyia spp., sh. 20–27. V. 97; 14–16. V. 98, 7–800 ft.

Visitors to female catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lapponicus F., sh. 18–20. V. 97, 800 ft. (2) B. terrestris L., sh. 22. V. 97, 7. V. 98, 700 ft. **Diptera.** *Empidae*: (3) Rhamphomyia cinerascens Mg., sh. 20. V. 97, 8–900 ft. *Muscidae*: (4) Lucilia cornicina F., 7. V. 98, 700 ft. (5) Pollenia vespillo F., 20. V. 97, 900 ft. *Anthomyiidae*: (6) Anthomyia sulciventris Ztt., sh. 20. V. 97, 800 ft. (7 and 8) Anthomyia spp., 20–22. V. 97, 7–900 ft.

230. Salix phylicifolia, Linn. (including *S. nigricans*, Sm.) [Lit *Arct.* 7, 34, 36.]

Visitor to male catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lapponicus F., sh. 13. V. 98, 700 ft.

Visitors to female catkins. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lapponicus F., sh. 13. V. 98, 800 ft. **Diptera.** *Syrphidae*: (2) Platychirus discimanus Lw., 21. V. 97, 1,300 ft. *Empidae*: (3) Rhamphomyia segmentata Ztt., sh. 13. VI. 99, 1,400 ft. *Chironomidae*: (4) 1 sp., 13. V. 98, 700 ft. *Anthomyiidae*: (5) Hyetodesia semicinerea W., sh. 13. V. 98, 7–800 ft. (6) Anthomyia sulciventris Ztt., 21. V. 97, 8–1,300 ft. (7 and 8) Anthomyia spp., 13. V. 98, 700 ft. *Cordyluridae*: (9) Scatophaga stercoraria L., 21. V. 97, 1,300 ft. **Coleoptera.** (10) Telephorus obscurus L., sh. 13. VI. 99, 1,400 ft.

231. Salix herbacea, Linn. [Lit. *Arct.* 36, 38; *Alps* 2, 9.] Fruits very freely.

Visitors to male catkins. **Hymenoptera.** Petiolata parasitica. *Ichneumonidae*: (1) 1 sp. Sessiliventres. *Tenthredinidae*: (2) Nematus ? capreae L., sh. **Diptera.** *Chironomidae*: (3 and 4) Ceratopogon spp., sh. *Anthomyiidae*: (5) Anthomyia sulciventris Ztt., sh. (6) Anthomyia sp. All 24. V. 97, 2,600 ft.

Visitors to female catkins. **Hymenoptera.** Petiolata parasitica. *Ichneumonidae*: (1) A second individual of the same species that was caught on the male catkins, sh. **Diptera.** *Anthomyiidae*: (2) Anthomyia sp., as named above. Both 24. V. 97, 2,600 ft.

TABLE XL.

Actual number of Individuals visiting the different species in Class AB.

	Apis.	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
184. Listera cordata . . .	—	—	—	—	1	—	—	—	—	—	—	2	3	—	6
185. Malaxis paludosa . . .	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
186. Oxalis Acetosella . .	—	—	—	—	—	—	—	—	1	—	3	9	—	—	13
187. Linum catharticum . .	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
188. Ribes Grossularia . .	5	4	—	—	—	—	1	—	—	—	1	6	—	1	18
189. Ribes rubrum	—	—	—	—	—	—	—	—	—	—	—	7	—	1	8
190. Berberis Aquifolium . .	—	2	—	—	—	—	—	—	—	—	—	—	—	—	2
191. Acer Pseudo-platanus .	15	13	—	—	1	—	5	2	—	—	—	41	31	—	108
192. Caltha palustris . . .	—	—	—	—	3	—	—	—	—	—	16	799	11	6	835
193. Vaccinium Oxycoccos .	—	—	—	—	1	—	—	—	—	—	—	1	—	—	2
194. Barbarea vulgaris . . .	—	—	—	—	—	—	—	—	—	—	—	—	5	—	5
195. Cochlearia officinalis .	—	—	—	—	—	—	—	1	—	—	—	10	2	1	14
196. Brassica Sinapis . . .	—	—	—	—	—	—	—	2	—	—	1	—	5	—	8
197. Capsella Bursa-pastoris .	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
198. Iberis amara	—	—	—	—	—	—	—	2	—	—	33	29	3	—	67
199. Trollius europaeus . .	—	—	—	—	1	—	—	—	—	—	1	26	8	—	36
200. Philadelphus coronarius	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1
201. Sedum Rhodiola . . .	—	—	—	—	3	—	—	—	—	—	2	16	8	—	29
202. Pyrus intermedia . . .	—	—	—	—	2	—	—	—	—	—	3	18	6	—	29
203. Pyrus Malus	—	—	—	—	—	—	—	—	—	—	—	14	22	—	36
204. Ranunculus Auricomus .	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
205. Ranunculus Flammula .	—	—	—	—	—	—	—	1	—	1	6	62	15	—	85
206. Ranunculus bulbosus .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
207. Ranunculus acris . . .	—	1	1	26	9	—	—	2	—	1	48	1414	131	6	1639
208. Ranunculus repens . .	—	—	—	3	—	—	—	—	—	—	3	98	19	—	123
209. Ranunculus Ficaria . .	—	—	—	—	—	—	—	—	—	—	—	35	—	1	36
210. Fragaria vesca	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
211. Potentilla Fragariastrum	—	—	—	—	2	—	—	—	—	—	—	6	—	—	8
212. Potentilla Tormentilla .	—	1	6	—	14	17	—	4	1	5	19	286	8	1	362
213. Potentilla maculata . .	—	—	—	—	—	—	—	—	—	—	1	3	—	1	5
214. Potentilla Comarum .	—	—	1	—	1	—	—	—	—	—	3	22	6	—	33
215. Parnassia palustris . .	—	—	—	—	3	4	—	—	—	—	—	11	7	10	35
216. Saxifraga nivalis . . .	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
217. Saxifraga hypnoides . .	—	—	—	1	10	—	—	1	1	—	12	73	48	5	151
218. Cerastium triviale . .	—	—	—	—	4	—	—	—	—	—	2	11	—	2	19
219. Cerastium alpinum . .	—	—	—	—	1	—	—	—	—	—	—	4	1	2	8
220. Stellaria media . . .	—	—	—	—	6	—	—	—	—	—	1	7	3	25	42
221. Stellaria Holostea . .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
222. Stellaria graminea . .	—	—	—	—	—	—	—	—	—	—	1	1	—	—	2
223. Sagina procumbens . .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
224. Spergula arvensis . . .	—	—	—	—	—	—	—	—	—	—	16	4	—	2	22
225. Loiseleuris procumbens .	—	—	—	—	—	—	—	—	—	—	—	6	—	—	6
Class AB. Section Ne.															
226. Salix Caprea	—	23	—	—	—	—	—	—	—	—	15	24	—	1	63
227. Salix aurita	47	67	1	—	—	1	1	—	—	—	49	79	1	—	246
228. Salix Lapponum . . .	—	1	—	—	—	—	1	—	—	—	—	13	—	—	15
229. Salix repens	1	6	—	1	—	—	—	—	—	—	25	32	—	—	65
230. Salix phyllicifolia . .	—	3	—	—	—	—	—	—	—	—	3	21	1	—	28
231. Salix herbacea	—	—	—	1	2	—	—	—	—	—	—	10	—	—	13
Total	68	121	9	32	66	22	8	16	3	7	264	3210	344	65	4235
Percentage	1·37	2·88	·21	76	1·56	·52	·19	·28	·07	·16	6·24	75·79	8·12	1·54	

TABLE XLI.

To show how class AB obtains visits from less than its share in proportion of decidedly desirable insects and more of indifferent insects.

	Available.		*To Class AB.*	
	No.	%	*No.*	%
Decidedly desirable	1,763	10·19	205	4·84
Desirable	1,277	7·37	276	6·52
Indifferent . . .	12,993	75·08	3,568	84·27
Injurious	1,273	7·36	185	4·37

TABLE XLII.

Insects visiting class AB in different seasons classed by desirability.

	Spring.		*Summer.*		*Autumn.*	
Decidedly desirable	188	16·33	16	·59	1	·27
Desirable	99	8·60	159	5·85	18	4·89
Indifferent . . .	848	73·73	2,421	89·17	299	81·02
Injurious	15	1·30	119	4·38	51	13·82
Total . . .	1,150		2,715		369	

TABLE XLIII.

Percentage of different groups of insects visiting Class AB in different seasons.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Spring	5·91	10·43	·07	·86	·17	—	—	—	8·52	72·26	1·30	·43
Summer	—	—	·29	3·64	·22	·58	·11	·25	5·45	79·11	9·57	·73
Autumn	—	·27	—	2·98	—	—	—	—	4·87	62·33	18·69	10·84

TABLE XLIV.

Desirability of visitors to Class AB according to colour, in percentages.

	Rose and purple	*Yellow.*	*White.*	*Green.*
Decidedly desirable	—	·41	1·10	30·96
Desirable	—	3·31	16·08	16·23
Indifferent . . .	87·50	93·40	64·98	50·83
Injurious	12·50	2·87	17·84	1·98

TABLE XLV.

Percentage of different groups of insects visiting flowers of different colours in Class AB.

	Api.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Rose-purple	—	—	—	12·33	—	—	—	—	—	87·56	—	—
Yellow . .	—	·12	·22	2·39	—	·28	·03	·22	3·06	86·58	6·59	·47
White . .	—	—	—	7·48	—	1·10	·44	—	15·63	44·71	20·26	10·35
Green . .	11·25	19·37	·33	1·49	1·32	·33	—	—	15·89	42·54	6·95	·49

In Class AB, Tables XLIV and XLV show that green flowers get the best visitors, and white flowers get, on the whole, better visitors than yellow flowers. In Table XLII it is seen that in spring many high types of insects go to AB. This is due to the frequent visits of Apis and Bombûs to *Acer Pseudo-platanus* and Salices—green flowers. If we take away *Salix* from Class AB, then we obtain Table XLVI:

TABLE XLVI.

Insects visiting Class AB (Salix excluded) in different seasons classed by desirability.

	Spring.		*Summer.*		*Autumn.*	
Decidedly desirable	40	5·46	16	·59	1	·27
Desirable	7	·95	158	5·83	18	4·89
Indifferent . . .	671	91·54	2,418	89·19	299	81·02
Injurious	15	2·05	119	4·39	51	13·82
Total . . .	733		2,711		369	

And if we also take away *Acer Pseudo-platanus* we leave hardly any insects of high type for spring.

The visitors to *Salix* which are, all but four, spring insects are:—

Decidedly desirable . .	148	34·34%
Desirable	93	21·58%
Indifferent	181	42·00%
Injurious	9	2·08%

Class AB attracted four species of the butterflies, four of the larger moths, and four of the smaller, including Eriocephala; of Hymenoptera, Apis, three species of Bombus, three of Andrena, two of Vespas, two ants, some eight or more species of parasitic Hymenoptera and four Tenthredinidae: among Diptera, in the Syrphidae, of one species of Sericomyia, two of Eristalis, three of Chilosia, four of Syrphus, a Chrysogaster, five of Platychirus, two of Melanostoma, two of Sphaerophoria, Syritta, and one of Ascia; in the Empidae of ten species of Empis, four of Rhamphomyia, two of Hilara and one of Clinoceira; in Tachinidae two species of Siphona; in

Muscidae, two species of Pollenia, one of Lucilia and one of Calliphora; in Sarcophagidae a Sarcophaga; in Cordyluridae three species of Scatophaga; in Sciomyzidae, one of Tetanocera, in Anthomyiidae twenty-three species including Drymia hamata; in Tipulidae two species; in Bibionidae two, in Dolichopodidae three; and of small flies seventeen; among Coleoptera eighteen species; among Hemiptera four species; among Neuroptera two species; among Thysanoptera Thrips; and among Araneida a crab-spider and a harvester spider.

CLASS Po. AB, § 62. ERECT AB-LIKE TYPE, WITH MUCH POLLEN.

232. Helianthemum vulgare, Linn. [Lit. *Brit.* 23, 89; *N.C.E.* 1, 3 b, 11, 33, Warnstorf 2507; *Alps* 2, 16, 34; *Pyren.* 17.]

Visitors. **Lepidoptera.** Heterocera: *Tortricidae*: (1) Tortrix sp. seeking h. 2. VI. 95, 800 ft. *Eriocephalidae*: (2) Eriocephala calthella L., fp. 21. VII. 95, 900 ft. **Hymenoptera.** Aculeata: *Apidae*: (3) Apis mellifica L., twice fp. 25. VI. 96, 700 ft. (4) Andrena analis Panz., 20. VI. 95, 1,000 ft. **Diptera.** *Syrphidae*: (5) Platychirus manicatus Mg., seeking h. 3. VII. 95; 19. VI. 99, 8–1,000 ft. (6) Chrysogaster hirtella Lw., fp. 18. VI. 96, 800 ft. (7) Sphaerophoria picta Mg., 3. VII. 95, 1,200 ft. (8) Ascia sp. fp. 25. VI. 96, 700 ft. *Mycetophilidae*: (9) Sciara sp. 4. VII. 94, about 800 ft. *Anthomyiidae*: (10) Hyetodesia lucorum Fln., fp. 29. VI. 95, 800 ft. (11) Hyetodesia sp., 4. VII. 94; 3. VII. 95, about 8–1,000 ft. (12) Limnophora sp., seeking h. and fp. 22. VI. 95, 800 ft. (13) Drymia hamata Fln., fp. 20. VI. 95, 1,000 ft. (14) Trichophthicus hirsutulus Ztt., fp. 24. VI.–23. VII. 95; 8. VII. 96, 8–1,200 ft. (15) Hylemyia nigrescens Rnd., 15–16. VI. 99, 800 ft. (16) Anthomyia radicum L., 4. VII. 94, about 800 ft. (17 and 18) Anthomyia spp. fp. 4. VII. 94; 14. VI.–3. VII. 95; 16. VI.–11. VII. 96; 19. VI. 99, 7–1,300 ft. (19) Homalomyia incisurata Ztt., 4. VII. 94, about 800 ft. (20) Coenosia sp., 4. VII. 94, about 800 ft. **Coleoptera.** (21) Meligethes viridescens F., 4. VII. 94, about 800 ft. **Neuroptera.** (22) Chloroperla sp., 4. VII. 94, about 800 ft.

233. Hypericum pulchrum, Linn. [Lit. *Brit.* 23; *N.C.E.* 14, 18.]

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Andrena analis Panz., 22. VI. 96, 1,000 ft. **Diptera.** *Syrphidae*: (2) Sphaerophoria sp., 28. V. 95, 1,000 ft. *Anthomyiidae*: (3) Hyetodesia incana W., fp. 27. VI. 95; 29. VI. 96, 11–1,800 ft. (4) Trichophthicus sp., fp. 5–17. VII. 95; 29. VI. 96, 8–1,500 ft. (5) Anthomyia sp., fp. 24. IX. 95, 900 ft.

234. Rosa mollis, Sm. (*R. villosa,* Linn.). [Lit. of *R. canina,* sensu ampliore, *Brit.* 23; *N.C.E.* 1, 3 b, 8, 14, 18, 34.]

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Bombus lapponicus F., seeking h. 10. VII. 96, 2,100 ft. (2) B. terrestris L., cp. and seeking h. 26. VI. 95; 26. VI. 96, 8–900 ft. **Diptera.** *Syrphidae*: (3) Eristalis rupium F., seeking h. 25–26. VI. 95, 800 ft. (4) E. arbustorum L., 8. VII. 95, 800 ft. *Anthomyiidae*: (5) Hyetodesia incana W., 26. VI. 96, 900 ft. (6) H. semicinerea W., 3. VII. 95, 800 ft. (7) Drymia hamata Fln., seeking h. 24. VI. 95, 800 ft. (8) Trichophthicus sp., fp. and

seeking h. 21. VI.–4. VII. 95, 800 ft. (9) Anthomyia sp., fp. and seeking h. 4–5. VII. 95; 24–25. VI. 96, 7–800 ft. **Thysanoptera.** (10) Thrips sp., 21. VI.–11. VII. 96, 7–800 ft. **Neuroptera.** (11) Chloroperla sp., 2. VII. 95, 800 ft.

CLASS Po. AB, § 63. AB-LIKE TYPE, WITH MASSED FLOWERS.

235. Spiraea Ulmaria, Linn. [Lit. *Brit.* 23, 39; *N.C.E.* 1, 3 b, 8, 11, 14 a, 16, 18, 21 a, 21 b, 33, 34, 40; *Arct.* 36; *Alps*, 2; *Pyren.* 17.]

Visitors. **Lepidoptera.** Heterocera: *Eriocephalidae*: (1) Eriocephala calthella L., fp. 6–17. VII. 95, 7–800 ft. **Hymenoptera.** Aculeata: *Apidae*: (2) Apis mellifica L., cp. and seeking h., 15–22. VII. 95; 3–11. VII. 96, 7–800 ft. freq. (3) Bombus lapponicus F., seeking h. 4–11. VII. 96, 800 ft. (4) B. terrestris L., cp. 15. VII. 95; 11. VII. 96, 7–800 ft. *Vespidae*: (5) Vespa norvegica F., seeking h. 4–11. VII. 96, 800 ft. Sessiliventres. *Tenthredinidae*: (6) Allantus arcuatus Forst., 29. V. 96, 900 ft. **Diptera.** *Syrphidae*: (7) Eristalis arbustorum L., fp. and seeking h., 15–17. VII. 95; 25. VI. 96, 7–800 ft. *Muscidae*: (8) Calliphora vomitoria L., 3. VII. 96, 800 ft. *Anthomyiidae*: (9) Hyetodesia incana W., fp. and seeking h., 15. VII. 95; 3–11. VII. 96, 800 ft. (10) Limnophora sp., 2. VII. 95, 800 ft. (11) Trichophthicus sp., fp. 12. VII. 95; 3. VII. 96, 7–800 ft. (12 and 13) Anthomyia spp., fp. 4–15. VII. 95; 11. VII. 96, 7–800 ft. **Cordyluridae**: (14) Scatophaga maculipes Ztt, fp. 15. VII. 95, 800 ft.

CLASS Po. AB, § 64. AB-LIKE TYPE WITH LITTLE POLLEN.

236. Lysimachia punctata, Linn. An escape from cultivation.

Visitor. **Diptera.** *Chironomidae*: (1) Ceratopogon flavipes Mg., seeking h., 2. VII. 95, 800 ft.

237. Lysimachia nemorum, Linn. [Lit. *Brit.* 23; *N.C.E.* 18, 33.]

Visitors. **Hymenoptera.** Aculeata: *Formicidae*: (1) Formica fusca Latr., seeking h., 29. VI. 96, 1,500 ft. **Diptera.** *Syrphidae*: (2) Syrphus sp., 23. VI. 96, 900 ft. *Chironomidae*: (3) Orthocladius sp., 29. VI. 95, 1,100 ft. *Anthomyiidae*: (4) 1 sp., fp. 10. VI. 99, 700 ft.

238. Trientalis europaea, Linn. [Lit. *N.C.E.* 1, 3 c, 4, 21 a, 21 b; *Arct.* 36.] The stamens and style are of equal length and are ripe together, but are not near enough to bring about spontaneous self-pollination.

Visitors. **Diptera.** *Syrphidae*: (1) Platychirus albimanus F., seeking h., 27. VI. 95, 2,100 ft. (2) Syrphus vitripennis Mg., fp. 15. VI. 99, 1,200 ft. *Empidae*: (3) Empis tessellata F., seeking h. 19. VI. 96, 1,500 ft. *Anthomyiidae*: (4) Anthomyia sp., fp. 22. V. 96; 10. VI. 99, 7–1,000 ft. **Coleoptera.** (5) Telephorus paludosus Fall., 20. VI. 95, 1,500 ft. **Thysanoptera.** (6) Thrips sp., 10. VI. 99, 700 ft.

239. Narthecium Ossifragum, Huds. [Lit. *Brit.* 23, 39; *N.C.E.* 3 a, 14, 14 a, 18; *Alps* 9.]

Visitors. **Lepidoptera.** Heterocera: *Eriocephalidae*: (1) Eriocephala calthella

L., fp. 28. VI.–6. VII. 95, 800 ft. **Hymenoptera.** Aculeata: *Apidae*: (2) Bombus hortorum L., seeking h., 11. VII. 96, once 800 ft. **Diptera.** *Syrphidae*: (3) Platychirus manicatus Mg., fp. 6. VII. 95, 900 ft. *Anthomyiidae*: (4) Hyetodesia incana W., fp. 6–11. VII. 96, 8–900 ft. (5) Hyetodesia sp., 2. VII. 95, 900 ft. (6) Trichophthicus sp., fp. 26. VI.–6. VII. 95; 1–6. VII. 96, 9–1,500 ft.

TABLE XLVII.

Total number of visitors to AB-like flowers of Class Po.

Class Po. (AB)	*Apis.*	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
232. Heliantheum vulgare .	2	—	1	—	—	—	—	—	1	1	8	55	—	—	68
233. Hypericum pulchrum .	—	—	1	—	—	—	—	—	—	—	1	15	—	—	17
234. Rosa mollis (villosa) .	—	5	—	—	—	—	—	—	—	—	3	17	—	3	28
235. Spiraea Ulmaria . . .	46	4	—	1	—	—	1	—	—	6	3	24	—	—	85
236. Lysimachia punctata .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
237. Lysimachia nemorum .	—	—	—	—	—	1	—	—	—	—	1	2	—	—	4
238. Trientalis europaea . .	—	—	—	—	—	—	—	—	—	—	3	12	1	1	17
239. Narthecium Ossifragum	—	1	—	—	—	—	—	—	—	11	1	16	—	—	29
Total	48	10	2	1	—	1	1	—	1	18	20	142	1	4	249
Percentages . . .	19·28	4·02	·80	·40	—	·40	·40	—	·40	7·23	8·03	57·03	·40	1·61	

Class A, § 65. Open Saxifraga Type.

240. Saxifraga aizoides, Linn. [Lit. *N.C.E.* 1; *Arct.* 7, 33, 37 b; *Alps* 2, 16; *Pyren.* 17.] Proterandrous with ultimate self-pollination. The stamens are of the usual two lengths, and go through the usual process of coming up singly to dehisce at the centre of the flower and finally coming up together to effect self-pollination.

Visitors. **Lepidoptera.** Heterocera: *Noctuidae*: (1) Hadena gemina Hb., sh. 18. VI. 96, 800 ft. *Tortricidae*: (2) 1 sp., sh. 22. VI. 96, 23–2,400 ft. *Eriocephalidae*: (3) Eriocephala calthella L., 28. VI.–11. VII. 96; 26. VI.–10. VII. 96, 800–2,400 ft. **Hymenoptera.** Aculeata: *Apidae*: (4) Bombus lapponicus F., sh. 1. VII. 95, 800 ft. and 1–10. VII. 96, 19–2,200 ft. (5) B. terrestris L., 14. IX. 95, 1,800 ft. (6) Andrena fucata Sm., sh. 23. VII. 95, 800 ft. *Vespidae*: (7) Vespa norvegica F., sh. 6–23. VII. 95; 4.–10. VII. 96, 800 ft. *Formicidae*: (8) Formica fusca Latr., sh. very ab. 21. VI.–23. VII. 95; 20–23. IX. 95; 23. VI.–10. VII. 96, 8–2,400 ft. *Myrmicidae*: (9) Myrmica rubra L., sh. freq. 22. VI.–11. VII. 95; 14–18. IX. 95; 5. VII. 96, 8–1,700 ft. Petiolata parasitica: *Ichneumonidae*: (10) Ichneumon molitorius Grav., sh. 18–24. IX. 95, 9–1,800 ft. (11) Hemiteles micator Grav., sh. 19. IX. 95, 2,400 ft. (12) Pimpla examinator F., 18. IX. 95, 800 ft. (13) Amblyteles subsericans Grav., sh. 4. IX. 95, 1,500 ft. (14, 15, 16, 17, 18, and 19) 6 spp. sh. 4. VII. 94; 1–23. VII. 95; 14–23. IX. 95; 18. VI.–10. VII. 96, 800–2,600 ft. *Chalcididae*: (20 and 21) 2 sp. sh. 22. VI.–10. VII. 96, 23–2,400 ft. *Cynipidae*: (22) 1 sp., 23. VII. 95, 800 ft. Sessiliventres: *Tenthredinidae*: (23) Tenthredo olivacea Klug., 6. VII. 95, 2,500 ft.

Diptera. *Syrphidae*: (24) Chilosia fraterna Mg., sh. 22. VI. 95; 8. VI. 96; 15. VI. 99, 8–1,100 ft. (25) Syrphus ? ribesii L., sh. 8. VI.–10. VII. 96, 8–2,300 ft. (26) S. ? vitripennis Mg., sh. 23. VII. 95, 800 ft. (27) Sphaerophoria scripta L., sh. 23. VII. 95, 800 ft. (28) Sericomyia borealis Fln., 2. VII. 96, 800 ft. (29) Eristalis arbustorum L., sh. 18. VI. 96, 800 ft. (30) Syritta pipiens L., sh. 18. VI.–10. VII. 96, 800 ft. *Empidae*: (31) Empis tessellata F., sh. 26. VI. 95; 2. VII. 96, 8-900 ft. (32) E. punctata Mg., sh. 23. VII. 95, 800 ft. (33) Rhamphomyia albosegmentata Ztt., 26. VI. 95, 1,000 ft. (34) R. ? sulcata Fln., 4. VII. 94, 800 ft. (35) Rhamphomyia sp., sh. 6. VII. 96, 800 ft. (36) Hilara matrona Hal., sh. 23. VII. 95, 800 ft. (37) Hilara sp., sh. 4. VII. 94; 4. VII. 95, 800 ft. *Mycetophilidae*: (38) Sciara sp., sh. 4–23. IX. 95; 1. VII. 96, 11–2,200 ft. *Bibionidae*: (39) Scatopse sp., sh. 6. VII. 96, 2,100 ft. (40) Dilophus albipennis Mg., 22. VI.–1. VII. 95; 26–27. VI. 96, 8–900 ft. (41) Bibio pomonae F., sh. 24. IX. 95; 10. VII. 96, 800 ft. *Simuliidae*: (42) Simulium sp., 25. VI. 96, 2,200 ft. *Chironomidae*: (43) Corynoneura sp., sh. 4. VII. 94, 18. VI. 96, 800 ft. (44) Ceratopogon sp., 4. VII. 94, 800 ft. *Psychodidae*: (45) Psychoda sp., 22. VI. 95, 11–1,800 ft. (46) Psychoda ?, 21. IX. 95, 1,100 ft. *Limnobidae*: (47) Limnophila meigenii Verrall, sh. 23. IX. 95, 1,400 ft. *Dolichopodidae*: (48) Dolichopus signatus Mg., sh. 28. VI. 95, 800 ft. (49) D. atratus Mg., sh. 4. VII. 94; 28. VI. 95, 800 ft. (50) D. pennatus Mg., sh. 28. VI. 95, 800 ft. (51) Dolichopus sp., sh. 18. VI.–6. VII. 96, 8–2,200 ft. (52) Argyra argentina Mg., sh. 23. VII. 95, 800 ft. *Tachinidae*: (53) Echinomyia fera L., sh. 1. VII. 95, 800 ft. *Sarcophagidae*: (54) Sarcophaga sp., 2. VII. 96, 800 ft. (55) Cynomyia mortuorum L., sh. 1–10. VII. 95, 8–2,200 ft. (56) C. alpina Ztt., 10. VII. 95, 2,200 ft. *Muscidae*: (57) Lucilia cornicina F., sh. 24. IX. 95, 17–1,800 ft. (58) Calliphora cognata Mg., sh. 10. VII. 95, 2,200 ft. (59) C. erythrocephala Mg., 18. VI.–6. VII. 96, 800 ft. (60) C. vomitoria L., sh. 4–23. VII. 95, 800 ft. (61) Pollenia rudis F., sh. 24. IX. 95, 1,800 ft. *Anthomyiidae*: (62) Hyetodesia incana W., sh. freq. 4. VII. 94; 22. VI.–23. VII. 95; 18. IX. 95; 18. VI.–4. VII. 96, 8–2,200 ft. (63) H. lucorum Fln., 4. VII. 94, 800 ft. (64) H. errans Mg., 4. VII. 94. (65) Limnophora solitaria Ztt., sh. 26. VI.–10. VII. 96, 18–2,300 ft. (66) Drymia hamata Fln., 25. VI. 95; 14. IX. 95, 800 ft. (67) Trichophthicus sp., sh. 6. VII. 95; 18. IX. 95, 8–1,300 ft. (68) Anthomyia sulciventris Ztt., 22. VI.–23. VII. 95, 8–900 ft. (69) A. radicum L., 4. VII. 94, 800 ft. (70, 71, and 72) Anthomyia spp., sh. 4. VII. 94; 6–13. VII. 95; 14–24. IX. 95; 19. VI.–10. VII. 96, 8–2,500 ft. (73) Coenosia sp., sh. 4. VII. 95, 800 ft. *Cordyluridae*: (74) Scatophaga stercoraria L., fp. sh. 4. VII. 94; 6. VII. 95; 14–23. IX. 95; 2–10. VII. 96, 8–2,500 ft. (75) S. squalida Mg., sh. 28. VI. 95, 800 ft. *Ortalidae*: (76) Pteropaectria frondescentiae L., 22. VI. 95; 18–22. VI. 96, 8–2,300 ft. *Sepsidae*: (77) Sepsis cynipsea L., 14. IX. 95, 1,500 ft. *Ephydridae*: (78) Hydrellia griseola Fln., sh. 28. VI. 95, 1,700 ft. *Chloropidae*: (79) Chloropisca ornata Mg., 1. VII. 95, 800 ft. *Phoridae*: (80) Phora rufipes Mg., sh. 22. VII. 95, 800 ft. (81) Phora sp., sh. 14–23. IX. 95, 8–1,800 ft. **Coleoptera.** (82) Meligethes viridescens F., sh. and fp. 28. VI. 95; 16–21. IX. 95; 26. VI.–6. VII. 96, 8–2,300 ft. (83) Sericosomus brunneus L., sh. 22. VI.–1. VII. 95, 9–1,100 ft. (84) Donacia discolor Panz., sh. 23. VII. 95, 800 ft. (85) D. sericea L., sh. 23. VII. 95, 800 ft. (86) Anthophagus alpinus Payk., sh. 6–13. VII. 95; 6. VII. 96, 20–2,400 ft. (87) Epuraea aestiva L., 23. IX. 95, 2,000 ft. (88) Telephorus pellucidus F., sh. 2. VII.

95, 800 ft. (89) Corymbites cupreus F., 4. VII. 94, 800 ft. **Araneida.** (90) Oligolophus morio F., sh. 23. IX. 95, 14–2,000 ft.

241. Saxifraga umbrosa, Linn. [Lit. *N.C.E.* Plateau 2002 ; *Alps* 2.] An escape from cultivation.

Visitors. **Hymenoptera.** Petiolata parasitica : (1) 1 sp. **Diptera.** *Syrphidae*: (2) Syritta pipiens L., sh. *Anthomyiidae*: (3) 1 sp. All 18. VI. 96, 900 ft.

242. Saxifraga stellaris, Linn. [Lit. *Brit.* 23 ; *Arct.* 7, 36, 37 a, 38 ; *Alps* 2, 21b.] At Clova the flower is variable, producing frequently extra parts ; the terminal flower is especially so, large, and often has three carpels (cf. Wydler in Flora, 1860, p. 387). This is partly in contrast to the observations of Warming and Schulz, who find the terminal flower is frequently female and small in size.

Visitors. **Diptera.** *Empidae*: (1) Empis lucida Ztt., 22. VI. 95, 2,600 ft. (2) E. vernalis Mg., ? sh. 12–16. VI. 99, 22–2,500 ft. *Anthomyiidae*: (3) Drymia hamata Fln., sh. 20. VI. 96, 2,300 ft. (4) Anthomyia sulciventris Ztt., 22. VI. 95 ; 21. V. 97, 10–1,800 ft. (5 & 6) Anthomyia spp., sh. 19. VI.–6. VII. 95 ; 23. V. 96 ; 20. VI.–6. VII. 96, 12–2,600 ft.

243. Chrysosplenium oppositifolium, Linn. [Lit. *Brit.* 23, 29 ; *N.C.E.* 1, 8 a.] Flowers ♀, by contabescence of the anthers, were observed once.

Visitors. **Lepidoptera.** Heterocera : (1) microlepidopteron, sh. 22. V. 96, 1,200 ft. **Hymenoptera.** Petiolata parasitica : *Ichneumonidae*: (2) 1 sp., 22. V. 97 ; 14. V. 98, 5–900 ft. **Diptera.** *Empidae*: (3) Empis tessellata F., sh. 22. V. 96, 1,200 ft. (4) Rhamphomyia sulcata Fln., sh. 22. V. 96, 1,200 ft. *Cecidomyiidae*: (5) Tanytarsus sp., sh. 14. V. 98 ; 15. VI. 99, 9–1,900 ft. *Mycetophilidae*: (6) Sciara sp., 20. VI. 95, 2,500 ft. *Psychodidae*: (7) Psychoda sp., sh. 14. V. 98, 900 ft. *Anthomyiidae*: (8) Anthomyia sulciventris Ztt., sh. 22. V. 97, 500 ft. (9) Anthomyia sp., 21. VI. 95, 1,400 ft. *Cordyluridae* : (10) Scatophaga stercoraria L., sh. 14. V. 98, 900 ft. (11) S. squalida Mg., 21. VI. 95, 1,400 ft. *Helomyzidae*: (12) Helomyza flava Mg., sh. 12. V. 98, 800 ft. **Coleoptera.** (13) Anthophagus alpinus Payk., 22. VI. 96, 2,400 ft. (14) Telephorus paludosus Fln., sh. 15. VI. 99, 1,900 ft. **Hemiptera.** (15) 1 sp., sh. 22. V. 97, 500 ft.

Class A, § 66. Aucuparia Type.

244. Pyrus aucuparia, Gaertn. [Lit. *Brit.* 23 ; *N.C.E.* 1, 3 b, 16, 18, 33, 40 ; de Vries 2460 ; *Pyren.* 17.)

Visitors. **Lepidoptera.** Heterocera : *Tineidae*: (1) 1 sp., 14. VI. 99, 1,400 ft. **Hymenoptera.** Aculeata : *Apidae*: (2) Apis mellifica L., sh. 11. VI. 99, 800 ft. (3) Bombus terrestris L., ran rapidly over an inflorescence ? sh., 16. VI. 99, 1,300 ft. (4) B. lapponicus F., 14. VI. 99, 1,400 ft. *Formicidae*: (6) Formica fusta Latr., 14–16. VI. 99, 1,400 ft. Petiolata tubulifera : *Chrysididae*: (6) Chrysis ? ignita L., 11. VI. 99, 800 ft. Petiolata parasitica : *Chalcididae*: (7) 1 sp., 14. VI. 99, 1,400 ft.

Diptera. *Syrphidae*: (8) Syrphus vitripennis Mg., sh. 11–16. VI. 99, 8–1,400 ft. (9) Chrysogaster hirtella Lw., 13. VI. 99, 1,400 ft. (10) Eristalis arbustorum L., 13–1,400 ft. *Empidae*: (11) Empis tessellata F., 16. VI. 99, 13–1,400 ft. (12) E. bilineata Lw., 11. VI. 99, 800 ft. (13) E. opaca F., 13. VI. 99, 1,400 ft. (14) E. lucida Ztt., 13–16. VI. 99, 13–1,400 ft. (15) Rhamphomyia sulcata Fln., sh. 11. VI. 99, 800 ft. (16) R. cinerascens Mg., sh. 11. VI. 99, 800 ft. *Chironomidae*: (17) 1 sp., 28. VI. 95, 1,800 ft. *Bibionidae*: (18) Scatopse sp., 14. VI. 99, 1,400 ft. (19) Bibio nigriventris Hal., 13–14. VI. 99, 13–1,400 ft. *Sarcophagidae*: (20) Sarcophaga sp., 13. VI. 99, 1,400 ft. *Muscidae*: (21) Lucilia sp., sh. 13–14. VI. 99, 13–1,400 ft. (22) Calliphora erythrocephala Mg., 11–16. VI. 99, 13–1,400 ft. (23) C. vomitoria L., 14. VI. 99, 1,400 ft. *Anthomyiidae*: (24) Mydaea sp., 11–16. VI. 99, 8–1,400 ft. (25) Limnophora sp., 28. VI. 95, 1,800 ft. (26) Drymia hamata Fln., sh. 16. VI. 99, 1,400 ft. (27) Trichophthicus sp., sh. 25. VI. 95, 2,000 ft. *Chloropidae*: (28) Chlorops sp., 14. VI. 99, 1,400 ft. **Coleoptera.** (29) Meligethes viridescens F., sh. 25–28. VI. 95; 11–14. VI. 99, 8–2,000 ft. (30) Epuraea aestiva L., sh. 25. VI. 95, 2,000 ft. (31) Corymbites cupreus F., 14. VI. 99, 1,400 ft. (32) C. quercus, Gyll., sh. 14. VI. 99, 13–1,400 ft. (33) Rhagium inquisitor F., devouring the anthers, 13–14. VI. 99, 1,400 ft. **Thysanoptera.** (34) Thrips sp., sh. 25. VI. 95; 14. VI. 99, 14–2,000 ft.

245. Crataegus Oxyacantha, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 b, 4, 14, 14 a, 16, 18, 34, 40; *Pyren.* 17.] Planted above 500 ft.

Visitors. **Hymenoptera.** Aculeata: *Apidae*: (1) Apis mellifica L., sh. 11. VI. 99, 800 ft. Petiolata parasitica: *Proctotrypidae*: (2) 1 sp., 11. VI. 99, 800 ft. **Diptera.** *Syrphidae*: (3) Eristalis arbustorum L., sh. 19. VI. 99, 700 ft. *Empidae*: (4) Empis bilineata Lw., 11. VI. 99, 800 ft. (5) Rhamphomyia sulcata Fln., sh. 11–12. VI. 99, 800 ft. *Bibionidae*: (6) Scatopse brevicornis Mg., sh. 11–12. VI. 99, 800 ft. **Coleoptera.** (7) Meligethes viridescens F., sh. 11–19. VI. 99, 7–800 ft.

Class A, § 67. Alchemilla Type.

246. Alchemilla alpina, Linn. [Lit. *N.C.E.* 1, 4, 34; *Alps* 2; *Pyren.* 17.] Very abundant.

Visitors. **Lepidoptera.** Rhopalocera: (1) Erebia epiphron Kn., 25. V. 95, once. Heterocera: *Noctuidae*: (2) Celaena haworthii Cuc., sh. 25. V. 95; 21. IX. 95, 18–2,000 ft. *Geometridae*: (3) Larentia salicata Hb., 15–21. VI. 95, 13–1,500 ft. *Crambidae*: (4) Pyrausta alpinalis Schiff., sh. 16–21. VI. 95, 17–2,300 ft. *Tortricidae*: (5) Tortrix sp., sh. 19–27. VI. 95, 17–2,400 ft. (6) Glyphipteryx fuscoviridella Haw., sh. 16. IX. 95, 900 ft. *Tineidae*: (7) Plutella cruciferarum Zel., sh. 19. VI. 95, 2,400 ft. **Hymenoptera.** Aculeata: *Formicidae*: (8) Formica fusca Latr., 16. VI.–1. VII. 95; 24. IX. 95; 19–26. VI. 96; 14. VI. 99, 9–2,100 ft. *Myrmicidae*: (9) Myrmica rubra L., sh. 16–21. IX. 95, 9–1,300 ft. Sessiliventres: *Tenthredinidae*: (10) Tenthredo olivacea Klug., 25. VI. 95, 2,000 ft. (11) Tenthredopsis sp., sh. 17. VI. 99, 1,100 ft. (12) Dolerus elongatus Thomson, sh. 15–25. VI. 95; 22. VI. 96, 12–2,400 ft. Petiolata parasitica: *Ichneumonidae*: (13) Ichneumon molitorius Grav., sh. 18–24. IX. 95, 9–2,400 ft. freq. (14) Alomyia debellator F., sh. 28. VI.–2.

VII. 95; 21–23. IX. 95, 8–1,800 ft. (15) Pimpla examinator F., sh. 24. IX. 95, 12–1,400 ft. (16) Limneria sp., sh. 21. IX. 95. 1,300 ft. (17) Limneria sp., sh. 21–23. IX. 95, 2,200 ft. (18) Polyrrhembia tenebricosa Grav., sh. 21. IX. 95, 1,600 ft. (19) Hemiteles politus Bridgman, 21. IX. 95, 1,700 ft. (20) H. micator Grav., 19. IX. 95, 2,300 ft. (21) Hemiteles sp., 15–20. VI. 95; 18–21. IX. 95, 8–1,600 ft. (22) Exephanes hilaris Grav., 19–23. IX. 95, 15–2,500 ft. (23) Campoplex sp., sh. 21. VI.–3. VII. 95, 11–2,200 ft. (24) Lissonotus sp., 25. VI. 95, 2,200 ft. (25) Xylonomus ?, sh. 20–23. IX. 95, 25–3,600 ft. (26) Pezomachus sp., 23. IX. 95, 1,700 ft. (27 and 28) Two other spp., 19. VI.–3. VII. 95; 20. IX. 95, 9–2,500 ft. *Brachonidae*: (29 and 30) Two spp., sh. 20. VI. 95; 16–24. IX. 95, 8–1,300 ft. *Proctotrypidae*: (31) Proctotrypes sp., 24. IX. 95, 1,200 ft. *Chalcididae*: (32, 33, 34, 35, 36, and 37) Six spp., sh. 16–20. VI. 95; 14–23. IX. 95; 18–30. VI. 96, 8–2,500 ft. *Cynipidae*: (38) Eucoela sp., 14–23. IX. 95, 17–1,800 ft. **Diptera.** *Syrphidae*: (39) Syrphus sp., 10. VII. 96, 2,300 ft. (40) Ascia podagrica F., 23. IX. 95, 2,300 ft. *Empidae*: (41) Empis tessellata F., sh. 21–25. VI. 95, 15–2,000 ft. (42) E. aestiva Lw., sh. 30. VI. 96, 2,100 ft. (43) E. lucida Ztt., 22. VI. 96, 2,400 ft. (44) Empis sp., 21. VI. 96, 1,300 ft. (45) Cyrtoma sp., sh. 3. VII. 95, 2,200 ft. (46) Hilara pilosa Ztt., sh. 24. VI. 95, 2,000 ft. (47) Tachydromia pubicornis Ztt., 16–23. IX. 95, 9–1,600 ft. (48) T. stigmatella Ztt., 3. VII. 95, 2,400 ft., and 4. VII. 94, 2,000 ft. *Cecidomyiidae*: (49) Cecidomyia sp., 4. VII. 94. (50) Lestremia sp., sh. 21–23. IX. 95, 12–2,000 ft. *Mycetophilidae*: (51) Sciara sp., sh. 16–24. VI. 95; 14–23. IX. 95; 1. VII. 96, 9–3,000 ft. freq. (52) Sceptonia nigra Mg., 4. VII. 94, ? 2,000 ft. (53) Boletina ? dubia Staeg., 23. IX. 95, 2,100 ft. *Bibionidae*: (54) Dilophus albipennis Mg., sh. 21–25. VI. 95; 24. IX. 95; 27. VI. 96, 11–2,200 ft. (55) Bibio pomonae F., sh. 23. IX. 95, 12–1,800 ft. *Simuliidae*: (56) Simulium sp., 25. VI. 95; 16. IX. 95, 9–1,800 ft. *Chironomidae*: (57) Corynoneura sp., 19–20. IX. 95, 23–3,600 ft. (58) Chironomus sp., 20. VI. 95; 23. IX. 95, 18–1,900 ft. (59) Crichotopus sp., sh. 3. VII. 95, 2,200 ft. (60) Tanytarsus sp., 23. IX. 95, 700 ft. (61) Metriocnemis sp., 23. IX. 95, 1,400 ft. (62) Ceratopogon sp., 23. IX. 95, 1,700 ft. *Psychodidae*: (63) Psychoda sp., 16–23. IX. 95, 9–1,800 ft. *Limnobidae*: (64) Rhypholophus nodulosus Mcq., sh. 23. IX. 95, 1,700 ft. *Tipulidae*: (65) Tipula excisa Schum., sh. 16–25. VI. 95, 19–2,400 ft. *Dolichopodidae*: (66) Dolichopus rupestris Hal., 6. VII. 95, 2,600 ft. (67) D. atratus Mg., 4. VII. 95, ? 2,000 ft. (68) Gymnopternus celer Mg., 16. IX. 95, 900 ft. *Sarcophagidae*: (69) Cynomyia mortuorum L., 21. VI. 95; 24. IX. 95, 12–1,300 ft. *Muscidae*: (70) Lucilia cornicina F., sh. 19–24. IX. 95, 9–2,400 ft. (71) Calliphora erythrocephala Mg., 23–24. IX. 95, 9–1,800 ft. (72) Pollenia rudis F., 19–24. IX. 95, 8–2,500 ft. freq. *Anthomyiidae*: (73) Hyetodesia incana W., sh. 20–28. VI. 95; 23. IX. 95; 19. VI. 96; 14–19. VI. 99, 8–2,500 ft. (74) H. semicinerea W., sh. 16. IX. 95, 900 ft. (75) H. basalis Ztt., 21. VI. 95, 1,500 ft. (76) H. sp., 4. VII. 95, 2,000 ft. (77) Mydaea ? tincta Ztt., 25. VI. 95, 1,700 ft. (78) Spilogaster nigrivenis Ztt., sh. 27. VI. 96, 1,400 ft. (79) S. quadrum F., sh. 24. VI. 95, 1,500 ft. (80) Limnophora solitaria Ztt., 26. VI.–4. VI. 95; 19–27. VI. 96, 19–2,300 ft. (81) Drymia hamata Fln., 16–25. VI. 95; 16. VI. 96, 13–2,200 ft. (82) Trichophthicus cunctans Mg., 4. VII. 95, ? 8,000 ft. (83 and 84) Trichophthicus spp., sh. 24. VI. 95; 19. IX. 95, 20–2,200 ft. (85) Hylemyia nigrescens Rnd., sh. 24. VI. 95, 1,700 ft.

(86) Anthomyia sulciventris Ztt., 27. VI. 95, 2,100 ft. (87, 88, and 89) Anthomyia spp., sh. 16. VI.–4. VII. 95; 21–24. IX. 95; 18. VI.–8. VII. 96, 8–2,700 ft. (90) Azelia aterrima Mg., 17. VI. 95, 2,200 ft., and ? 4. VII. 95. (91) Caricea sp., 19. VI. 95, 2,400 ft. (92) Coenosia infantula Rnd., 1. VII. 95, 1,200 ft. (93) Coenosia sp., sh. 24. VI. 95, 1,600 ft. *Cordyluridae*: (94) Scatophaga stercoraria L., ? fp. 19. VI.–6. VII. 95; 16–24. IX. 95, 10–2,700 ft. (95) S. squalida Mg., 20–21. VI. 95, 13–1,900 ft. *Sciomyzidae*: (96) Tetanocera sp., 16. IX. 95, 900 ft. *Ortalidae*: (97) Pteropaectria frondescentiae L., 1. VII. 96, 1,900 ft. *Sepsidae*: (98) Sepsis cynipsea L., 23. IX. 95, 16–1,900 ft. *Ephydridae*: (99) Hydrellia griseola Fln., 3. VII. 95, 2,600 ft. *Chloropidae*: (100) Chlorops sp., 3. VII. 95, 2,600 ft. *Borboridae*: (101) Borborus equinus Fln., 23. IX. 95, 1,800 ft. *Phoridae*: (102) Phora rufipes Mg., 14. IX. 95, 1,200 ft. (103) Phora sp., sh. 16. VI.–3. VII. 95; 16–24. IX. 95, 8–2,400 ft. freq. **Coleoptera.** (104) Meligethes viridescens F., sh. 24. VI. 95, 1,600 ft. (105) Anthophagus alpinus Payk., sh. 3–4. VII. 95; 22. VI.–6. VII. 96, 24–2,700 ft. (106) Epuraea aestiva L., 3. VII. 95, 2,500 ft. (107) Corymbetes cupreus F., 26. VI. 95, 1,000 ft. (108) C. quercus Gyll., 16–28. VI. 95, 17–2,000 ft. (109) Dolopius marginatus L., 4. VII. 94. (110) Helodes marginata F., 15. VI. 95, 1,200 ft. (111) Sericosomus brunneus L., 25. VI. 95, 1,700 ft. (112) Telephorus paludosus Fln., 16.–20. VI. 95. (113) Rhagonycha limbata Thoms., sh. 17–24. VI. 95; 19. VI. 96, 12–2,000 ft. (114) Malthodes atomus Thoms., 18. VI. 96, 1,100 ft. (115) Apion sp., 16. IX. 95, 900 ft. **Hemiptera.** (116) Heterocordylus tibialis Hahn., 25. VI. 95, 800 ft. (117) Nabis flavomarginatus Scholtz, 21–23. IX. 95, 16–1,800 ft. (118) Stygnus sp., 21. IX. 95, 1,700 ft. (119) Psylla ?, sh. 21. IX. 95, 1,800 ft. **Neuroptera.** (120) 1 sp., 20. VI. 95, 1,700 ft. **Collembola.** (121) One springtail, sh. 16–23. IX. 95, 9–1,500 ft. **Araneida.** (122) Xysticus?, lying in wait, 23. IX. 95, 1,700 ft. (123) Oligolophus morio F., sh. 21–2. IX. 95, 10–2,300 ft.

247. Alchemilla vulgaris, Lam. [Lit. *Brit.* 23; *N.C.E.* 1, 4, 9, 14, 21 b, Plateau 2002; *Arct.* 36; *Alps* 2, 16; *Pyren.* 17.] This begins to flower before *A. alpina.*

Visitors. **Lepidoptera.** Rhopalocera: (1) Coenonympha pamphilus L., 15. VI. 95, 800 ft. Heterocera: *Geometridae*: (2) Larentia salicata Hb., 20. VI. 95, 2,400 ft. **Hymenoptera.** Petiolata parasitica: *Ichneumonidae*: (3, 4, and 5) 3 spp., sh. 15. VI. 95; 16–22. IX. 95; 27. V. 97, 7–800 ft. *Chalcididae*: (6) 1 sp., 16–17. IX. 95, 800 ft. **Diptera.** *Empidae*: (7) Empis lucida Ztt., 15. VI. 95, 800 ft. *Mycetophilidae*: (8) Sciara sp., sh. 22. IX. 95, 800 ft. *Chironomidae*: (9) Ceratopogon leucopeza Mg., 22. IX. 95, 800 ft. (10) A second sp., 13. V. 98, 800 ft. *Tachinidae*: (11) Gymnochaete viridis Fln., sh. 22. V. 96, 800 ft. *Muscidae*: (12) Pollenia rudis F., sh. 15–22. IX. 95, 800 ft. *Anthomyiidae*: (13) Hyetodesia incana W., 15–17. VI. 95; 15. IX. 95, 800 ft. (14, 15, and 16) Anthomyia spp., sh. 17. VI. 95; 15–22. IX. 95; 19. V. 97; 15. V. 98, 8–2,400 ft. *Cordyluridae*: (17) Scatophaga stercoraria L., sh., 20. VI. 95; 15–22. IX. 95; 22. V. 96, 800, and once at 2,400 ft. *Psilidae*: (18) Psila?, 17. VI. 95, 800 ft. *Trichaetidae*: (19) Tephritis leontodontis, sh. 22. IX. 95, 800 ft. *Phoridae*: (20) Phora sp., 15. IX. 95, 800 ft. **Coleoptera.** (21) Meligethes viridescens F., 16. IX. 95, 800 ft. (22) Enicmus minutus L., 22. IX. 95,

800 ft. **Neuroptera.** (23) Chloroperla sp., 21. VI. 95, 800 ft. **Hemiptera.** (24) Aphis sp., 17. VI. 95, 800 ft.

Class A, § 68. Galium Type.

248. Galium verum, Linn. [Lit. *Brit.* 23; *N.C.E.* 1, 3 c, 9, 14, 14 a, 18, 21 a, 31, 32, 40; *Pyren.* 17.]

Visitors. **Lepidoptera.** Heterocera: *Eriocephalidae*: (1) Eriocephala calthella L., sh. and fp. 4–20. VII. 95, 800 ft. **Hymenoptera.** Sessiliventres: *Tenthredinidae*: (2) Allantus arcuatus Forst., 5. VII. 95, 800 ft. (3) Abia sp., 6. VII. 95, 800 ft. Petiolata parasitica: *Ichneumonidae*: (4) 1 sp., 2. VII. 95, 800 ft. (5) 10. VII. 96, 900 ft. **Diptera.** *Syrphidae*: (6) Syritta pipiens L., sh. 23. VII. 95, 800 ft. *Mycetophilidae*: (7) Sciara sp., 16. IX. 95, 800 ft. *Bibionidae*: (8) Dilophus albipennis Mg., sh. 3. VII. 95, 800 ft. (9) Bibio pomonae F., 10–11. VII. 96, 8–900 ft. *Muscidae*: (10) Lucilia cornicina F., sh. 7. VII. 95, 800 ft. (11) Calliphora erythrocephala Mg., sh. 5–23. VII. 95; 11. VII. 96, 800 ft. (12) Mesembryna meridiana L., sh. 10. VII. 96, 800 ft. *Anthomyiidae*: (13) Hyetodesia incana W., sh. 3–23. VII. 95; 1–11. VII. 96, 7–900 ft. (14) Trichophthicus hirsutulus Ztt., sh. 10. VII. 96, 900 ft. (15) Anthomyia sp., sh. 10. VII. 96, 900 ft. *Sepsidae*: (16) Sepsis cynipsea L., 5. VII. 95, 800 ft. *Chloropidae*: (17) Oscinis sp., 5. VII. 95, 800 ft. **Coleoptera.** (18) Meligethes aeneus F., sh. 3. VII. 95, 800 ft. (19) Corymbites quercus Gyll., 2. VII. 95, 800 ft. (20) Serica brunnea L., 2. VII. 95, 800 ft. (21) Thyamis laevis Duft., 5. VII. 95, 800 ft.

249. Galium boreale, Linn. [Lit. *N.C.E.* 1, 3 c, 21 a, 34; *Alps* 2.] Proterandrous, the stigmas not receptive until the anthers have dehisced and the stamens have bent back out of the way between the petals. This they do on the third day after the opening of the bud.

Visitors. **Lepidoptera.** Heterocera: *Crambidae*: (1) Pyrausta ? alpinalis Schiff., sh. 10. VII. 96, 2,000 ft. **Hymenoptera.** Aculeata: *Formicidae*: (2) Formica fusca Latr., sh. 6. VII. 95, 1,800 ft. Petiolata parasitica: (3) 1 sp., 25. VI. 96, 700 ft. **Diptera.** *Syrphidae*: (4) Platychirus manicatus Mg., sh. 25. VI. 96, 700 ft. (5) Syritta pipiens L., sh. 11. VII. 96, 700 ft. *Bibionidae*: (6) Bibio pomonae F., sh. 11. VII. 96, 700 ft. *Orphnephilidae*: (7) Orphnephila testacea Ruthé, 10. VII. 96, 2,000 ft. *Anthomyiidae*: (8) Hyetodesia variabilis Fln., sh. 25. VI. 96, 700 ft. (9) Spilogaster nigrivenis Ztt., sh. 25. VI. 96, 700 ft. (10) Limnophora solitaria Ztt., sh. 26. VI.–10. VII. 96, 19–2,100 ft. (11) Drymia hamata Fln., sh. 15. VII. 95, 2,400 ft. (12) Anthomyia sulciventris Ztt., 25. VI. 96, 700 ft. (13) A. sp., 6. VII. 96, 1,700 ft. *Cordyluridae*: (14) Scatophaga sp., sh. 25. VI. 96, 700 ft.

250. Galium saxatile, Linn. (with *G. sylvestre* Poll.). [Lit. *N.C.E.* 1, 3 c, 4, 14, 21 a, 21 b, 32, 33; *Alps* 2, 21 b.]

Visitors. **Lepidoptera.** Rhopalocera: (1) Argynnis aglaia L., sh. 25. VI. 95, 800 ft. (2) Coenonympha pamphilus L., 25–28. VI. 95, 8–15,100 ft. Heterocera: *Geometres*: (3) Psodos trepidaria Tr., sh. 29. VI. 95, 2,500 ft. **Hymenoptera.**

Petiolata parasitica: *Proctotrypidae*: (4) Proctotrype sp., sh. 20. IX. 95, 3,700 ft. **Diptera.** *Empidae*: (5) Empis tessellata F., 1. VII. 95, 800 ft. once. *Psychodidae*: (6) Psychoda ?, sh. 22. IX. 95, 3,600 ft. *Muscidae*: (7) Lucilia cornicina F., 26. VI. 95, 800 ft. twice. (8) Calliphora vomitoria L., 3. VII. 95, 800 ft. once. (9) Pollenia rudis F., 16. VI. 95, 800 ft. once. *Anthomyiidae*: (10) Hyetodesia incana W., 26. VI. 95, 800 ft. (11) Limnophora solitaria Ztt., sh. 26. VI. 96, 2,200 ft. (12) Drymia hamata Fln., 25. VI.–3. VII. 95; 29. VI. 96, 8–2,600 ft. (13) Trichophthicus sp., ? sh. 2. VII. 96, 2,800 ft. (14) Anthomyia sp., sh. 15–21. VI. 95; 10. VI.–8. VII. 96, 800 ft. *Cordyluridae*: (15) Scatophaga stercoraria L., sh. 21. VI. 95; 24. VI. 96, 800 ft. *Ephydridae*: (16) Hydrellia griseola Fln., 3. VII. 95, 1,600 ft. *Chloropidae*: (17) Chlorops ?, 10. VII. 96, 2,200 ft. (18) Oscinis sp., 21. VII. 95, 800 ft. **Coleoptera.** (19) Corymbites cupreus F., sh. 19. VI. 96, 900 ft.

251. Galium Aparine, Linn. [Lit. *Brit.* 23; *N.C.E.* 18, 33, 34.]

Visitors. **Hymenoptera.** Petiolata parasitica: *Chalcididae*: (1) 1 sp., sh. 1–6. VII 95. **Diptera.** *Anthomyiidae*: (2) Coenosia sp., 1. VII. 95. Both at 800 ft.

252. Galium palustre, Linn. [Lit. *Brit.* 23; *N.C.E.* 8, 14, 18, 25, 34.]

Visitors. **Diptera.** *Empidae*: (1) Empis tessellata F., sh. 1. VII. 95. (2) E. stercorea L., sh. 1. VII. 95. *Muscidae*: (3) Lucilia cornicina F., sh. 1. VII. 95. *Anthomyiidae*: (4) Hyetodesia incana W., 2–6. VII. 95. (5) H. variabilis Fln., 1. VII. 95. All at 800 ft.

Class A, § 69. Arenaria sedoides Type.

253. Arenaria sedoides, Schultz. [Lit. *Alps* 2, 21 b.] The flower opens very widely, so as to expose the abundant honey freely. The stamens bend very far out after dehiscing; but, as here and there an anther does not fall off, self-pollination may be brought about in the closing of the flower. Its duration is three to four days. The yellow nectaries are the most conspicuous part of the flower. Seed is freely produced.

Visitors. **Hymenoptera.** Petiolata parasitica: (1) 1 sp., sh. 27. VI. 96. **Diptera.** *Mycetophilidae*: (2) Sciara sp., sh. 19. VI. 99, and ? 2. VII. 96. *Bibionidae*: (3) Bibio nigriventris, Hal., sh. covered with pollen, 16. VI. 99. *Anthomyiidae*: (4) Anthomyia sp., 16. VI. 99. *Cordyluridae*: (5) Scatophaga stercoraria L., sh. 16. VI. 99. *Sapromyzidae*: (6) Sapromyza sp., 16. VI. 99. **Coleoptera.** (7) 1 sp. similar to Amara bifrons Gyll., sh. 16. VI. 99. **Collembola.** (8) 1 sp., sh. 2. VII. 96. **Acarina.** (9) 1 sp., sh. 2. VII. 96. All at 2,850 ft.

Class A attracted three species of the butterflies, three of the larger moths, and six of the smaller moths, including Eriocephala; of Hymenoptera, Apis, two species of Bombus, one of Andrena, one of Vespa, two species of ant, five of Tenthredinidae, one of Chrysis, and about thirty of parasitic

TABLE XLVIII.

Actual number of Individuals visiting the flowers of Class A.

	Apis.	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
240. Saxifraga aizoides . . .	—	8	1	2	50	130	6	1	3	46	29	309	25	3	613
241. Saxifraga umbrosa . . .	—	—	—	—	1	—	—	—	—	—	1	2	—	—	4
242. Saxifraga stellaris . . .	—	—	—	—	—	—	—	—	—	—	4	8	—	—	12
243. Chrysosplenium oppositofolium	—	—	—	—	2	—	—	—	1	—	1	18	2	2	26
244. Pyrus Aucuparia . . .	1	2	1	—	1	3	—	—	1	—	33	110	79	51	282
245. Crataegus Oxyacantha .	1	—	—	—	1	—	—	—	—	—	2	5	4	—	13
246. Alchemilla alpina . . .	—	—	—	8	108	25	—	6	9	—	9	507	31	25	728
247. Alchemilla vulgaris . .	—	—	—	—	6	—	—	2	—	—	1	82	3	2	96
248. Galium verum	—	—	—	2	2	—	—	—	—	19	1	49	4	—	77
249. Galium boreale	—	—	—	—	1	1	—	—	2	—	2	25	—	—	31
250. Galium saxatile	—	—	—	—	3	—	—	4	—	—	1	29	1	—	38
251. Galium Aparine	—	—	—	—	3	—	—	—	—	—	—	2	—	—	5
252. Galium palustre	—	—	—	—	—	—	—	—	—	—	4	6	—	—	10
253. Arenaria sedoides . . .	—	—	—	—	1	—	—	—	—	—	—	17	1	4	23
Total	2	10	2	12	179	159	6	13	16	65	88	1169	150	87	1958
Percentages	·10	·51	·10	·61	9·15	8·13	·31	·66	·82	3·32	4·50	59·73	7·67	4·45	

TABLE XLIX.

To show how great a proportion of injurious insects go to Class A.

	Available.		*To Class A.*	
	No.	%	*No.*	%
Decidedly desirable	1,763	10·19	25	1·28
Desirable	1,277	7·37	106	5·41
Indifferent . . .	12,993	75·08	1,390	70·98
Injurious	1,273	7·36	437	22·32

TABLE L.

Insects visiting Class A in different seasons classed by desirability.

	Spring.		*Summer.*		*Autumn.*	
Decidedly desirable	—	—	22	1·66	3	·53
Desirable	2	3·03	93	7·18	9	1·58
Indifferent . . .	59	89·39	930	70·75	395	69·42
Injurious	5	7·57	270	20·41	162	28·47
Total . . .	66		1,323		569	

TABLE LI.

Percentages of different groups of insects visiting Class A in different seasons.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Anis.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Spring	—	—	—	4·54	—	—	1·51	—	1·51	89·39	—	3·03
Summer	·15	·60	·15	15·94	·45	·90	·52	4·91	6·50	54·72	10·65	4·45
Autumn	—	·35	—	23·90	—	·17	1·40	—	·17	67·83	1·58	4·56

TABLE LII.

Desirability of the visitors to flowers of Class A, according to colour, in percentages.

	Yellow.	*White.*	*Green.*
Decidedly desirable	1·26	2·03	·94
Desirable	5·03	12·91	2·24
Indifferent . . .	66·76	68·61	75·68
Injurious	26·95	16·45	21·13

TABLE LIII.

Percentages of different groups of insects visiting flowers of different colours in Class A.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Anis.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Yellow	—	1·11	·13	26·25	·83	·13	·55	9·07	4·32	52·52	4·32	·69
White.	·50	50	·25	3·44	—	1·01	·75	—	11·89	47·34	21·26	12·91
Green.	—	—	—	17·47	—	·94	1·06	—	1·18	71·54	4·13	3·65

Hymenoptera; among the Diptera, in Syrphidae, of one species of Eristalis, of Sericomyia, of Chilosia, and of Chrysogaster, of three species of Syrphus, and one of each of the following: Platychirus, Syritta, Sphaerophoria, and Ascia; in Empidae, of eight species of Empis, and of the same number of short-tongued Empids; in Tachinidae, only of Echinomyia and Gymnochaeta viridis, and not of Siphona; in Muscidae, of three species of Calliphora and one each of Lucilia, Mesembryna, and Pollenia; in Sarcophagidae, of two species of Cynomyia and one Sarcophaga; in Anthomyiidae, of twenty-seven species, including Drymia hamata; in Tipulidae, of two species; in Bibionidae, of four; in Cordyluridae, of two species of Scatophaga; and of thirty-two species of the smaller flies, including almost all the Dolichopids observed; among Coleoptera, of twenty species; among Hemiptera, of five species; among Neuroptera, of two; among Thysanoptera, of one; among Collembola, of one; in Acarina, of one; and in Araneida, of two.

A brief review of the insects visiting the massed flowers of Class A may be found earlier.

Class Po. A, § 70. A-like Type.

254. Chenopodium Bonus-Henricus, Linn. [Lit. *N.C.E.* 1, 14, 18, 21 b, 34; Warming 2490.

Visitor. **Coleoptera.** (1) Oxytelus sp., ? fp. 19. VI. 99, 800 ft.

The only visitors to Class Po. § A were two individuals of a beetle.

TABLE LIV.

Total number of insect visitors to A-like flowers of Class Po., and total of visitors to B-like, AB-like, and A-like flowers, taken together.

Class Po. (A).	*Apis.*	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
254. Chenopodium Bonus-Henricus	—	—	—	—	—	—	—	—	—	—	—	—	2	—	2
Total of all flowers of B-like, AB-like, and A-like Po. . .	56	14	2	1	1	1	1	—	1	18	23	344	5	6	473
Percentages . .	11·84	2·96	·42	·21	·21	·21	·21	—	·21	3·81	4·86	72·73	1·06	1·27	

TABLE LV.

Decidedly desirable insects visit honeyless flowers (for pollen) in rather more than the class's share; injurious insects are rare on them.

	Available.		*To Po. (all forms).*	
	No.	%	*No.*	%
Decidedly desirable	1,763	10·19	70	14·80
Desirable	1,277	7·37	26	5·50
Indifferent . . .	12,993	75·08	368	77·80
Injurious	1,273	7·36	9	1·90

TABLE LVI.

Insects visiting Class Po. (all three divisions taken together) in different seasons by desirability.

	Spring.		*Summer.*		*Autumn.*	
Decidedly desirable	12	5·50	58	23·67	—	—
Desirable	3	1·37	23	9·39	—	—
Indifferent . . .	203	93·11	155	63·27	10	100·00
Injurious	—	—	9	3·67	—	—
Total . . .	218		245		10	

TABLE LVII.

Percentages of different groups of insects visiting honeyless flowers (Class Po.) in the different seasons.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Spring .	3·67	1·84	—	—	—	—	—	—	1·38	95·12	—	—
Summer	19·59	4·08	·82	1·22	·41	—	·41	7·35	8·16	53·47	2·04	2·45
Autumn	—	—	—	—	—	—	—	—	—	100·00	—	—

TABLE LVIII.

Desirability of visitors to honeyless flowers (Class Po.) according to colour, in percentages.

	Rose-purple.	*Yellow.*	*White.*
Decidedly desirable	17·85	2·52	19·12
Desirable. . . .	10·71	11·76	2·77
Indifferent . . .	60·71	84·78	76·52
Injurious	10·71	·84	1·53

TABLE LIX.

Percentages of different groups of insects visiting honeyless flowers (Class Po.) of different colours.

	Apis.	*Bomb.*	*Hm.*	*Phyt. Entom. Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*
Rose-purple	—	17·85	—	—	—	—	—	—	10·71	60·71	—	10·71
Yellow . .	1·68	·84	1·68	·84	—	—	·84	10·08	9·24	74·70	—	—
White . .	16·66	2·46	—	·61	·30	—	—	1·85	2·77	73·45	·92	·92

Class Po. was visited by a Tortrix and by Eriocephala calthella; among Hymenoptera, by Apis, by three species of Bombus, by one of Andrena, one wasp, one ant, one Tenthredine, and one Chalcidid; among Diptera, in Syrphidae, by two species of Eristalis, by one each of Syrphus, Chrysogaster, Ascia, and Sphaerophoria, and by three of Platychirus; in Empidae, only by Empis tessellata, and then only on a single occasion; in Muscidae, by a Calliphora and a Lucilia; by thirteen species of Anthomyiidae; by two species of Scatophaga, a Dilophus, and by four other small flies; by three Coleoptera; one Neuroptera; and in Thrysanoptera, by Thrips.

CLASS W, § 71.

255. Plantago lanceolata, Linn. [Lit. *Brit.* 23, Darwin 485; *N.C.E.* 1, 3 a, 3 b, 3 c, 4, 8, 9, 10, 11, 12, 14, 18, 21 a, 21 b, 30, 33, 34.]

Visitor. **Hymenoptera.** Aculeata: *Vespidae*: (1) Vespa sylvestris Scop., seeking h. 4. VII. 95, 900 ft.

256. Urtica dioica, Linn. [Lit. *N.C.E.* 1, 3 a, 3 b, 3 c, 4, 8, 9, 10, 11, 12, 14, 18, 34.) Vegetative reproduction seems to continue uninterruptedly.

Visitors. **Diptera.** *Syrphidae*: (1) Chrysogaster hirtella Lw., sitting on flowers. 15. VII. 95, 800 ft. *Cordyluridae*: (2) Scatophaga stercoraria L., on flowers, 24. VI. 95, 800 ft. *Anthomyiidae*: (3) Azelia sp., fp. 21. VI. 95, 800 ft. **Coleoptera.** (4) Brachypterus urticae F., fp. 21.–24. VI. 95, 8–900 ft. freq.

257. Betula alba, Linn. [Lit. *N.C.E.* 14, 18.]

Visitor. **Diptera.** *Syrphidae*: (1) Syrphus punctulatus Verrall, fp. 10. V. 97 800 ft.

258. Carex dioica, Linn.

Visitors. **Diptera.** (1) One short-tongued fly, fp. **Coleoptera.** (2) One sp., fp. Both 22. V. 96, 1,100 ft.

259. Carex glauca, Murr.

Visitor. **Diptera.** *Anthomyiidae*: (1) Anthomyia sulciventris Ztt., fp. 22. V. 97, 600 ft.

260. Alopecurus pratensis, Linn. [Lit. *N.C.E.* 14, 18, 34.]

Visitor. **Diptera.** *Syrphidae*: (1) Platychirus manicatus Mg., fp. 15. VI. 99, 800 ft.

261. Abies excelsa, Poiret. [Lit. *N.C.E.* 34.]

Visitor. **Lepidoptera.** Rhopalocera: (1) Pieris napi L., once hovering about young female cones, apparently seeking h., 11. VI. 99, 900 ft.

TABLE LX.

Total number of individuals visiting wind-fertilized flowers, Class W.

	Apis.	*Bomb.*	*Hm.*	*Tenthr.*	*Entom.*	*Ants.*	*Wasps.*	*Lep. l.*	*Lep. m.*	*Lep. s.*	*Dm.*	*Ds.*	*Col.*	*Etc.*	*Total.*
255. Plantago lanceolata	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
256. Urtica dioica . . .	—	—	—	—	—	—	—	—	—	—	1	5	53	—	59
257. Betula alba . . .	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
258. Carex dioica . . .	—	—	—	—	—	—	—	—	—	—	—	1	2	—	3
259. Carex glauca . . .	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
260. Alopecurus pratensis	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
261. Abies excelsa . . .	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1
Total . . .	—	—	—	—	—	—	1	1	—	—	3	7	55	—	67
Percentage . .	—	—	—	—	—	—	1·49	1·49	—	—	4·48	10·45	82·09	—	—

The flowers of Class W deceived into visiting them one butterfly and one wasp; they also received the visits, among Diptera, of three Syrphids, a Scatophaga, an Anthomyia, and an Azelia; and among Coleoptera, of two species.

We abstain in this paper from comparing our results with those of Müller, MacLeod, and others; the comparison will follow in the last part of our paper, wherein too we shall discuss fully the results of our work.

A Study of the Vascular System in certain Orders of the Ranales.

BY

W. C. WORSDELL, F.L.S.

With Plates XXXII and XXXIII, and four Figures in the Text.

I

THE general arrangement of the vascular system in the vegetative organs, viz. the stems and leaves, of a plant is obviously directly correlated with, and the result of, the habit of the plant. Now, according to their habit, plants may be grouped into two main series:—

(1) Grandifoliate plants, in which the stem plays a very subsidiary rôle, the length of its internodes being reduced to a minimum; the leaves, therefore, closely succeed each other, possess wide sheathing bases, and are the dominant organs of the plant, e. g. Palms, Water Lilies.

(2) Parvifoliate plants, in which the stem is the dominant organ of the plant, possessing elongated internodes, and bearing comparatively small leaves with non-sheathing bases, e. g. Elm, Wall-flower.

In the former group, the vascular structure of the stem will be dominated by and similar to that of the leaf, in which the bundles will tend to be scattered throughout the ground-tissue; in the latter group the vascular structure of the stem will be much more independent of, and more unlike, that of the leaf, in which the bundles will tend to be arranged in an arc.

Taking the vegetable kingdom as a whole, I regard the grandifoliate habit as the primitive one, for the simple reason that I consider the bryophytic sporogonium as the prototype and ancestor of all plants; in this structure the vegetatively-modified *terminal* capsule was the primaeval leaf, dominating, as regards size and general development, the stem (seta) immediately below it. Out of this developed the subsequent leafy plant, by a process of sympodial and lateral branching of phytons ('Stengelglieder'), precisely as occurs in the embryo and seedling of,

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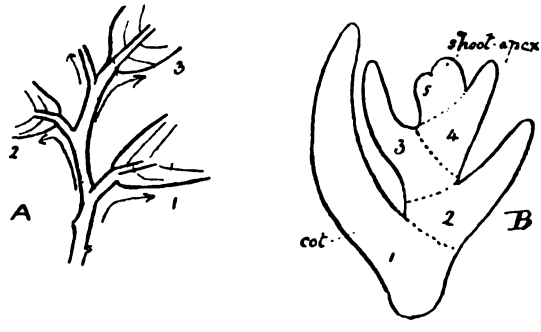
e. g. *Juncus* and *Pistia* at the present day, each branch bearing a terminal leaf, until such period as the stem-apex began precociously to assert itself at each stage and the leaves became displaced from their primitively terminal position into a lateral one. This process is repeated in the embryonic history of the great majority of Monocotyledons and is the explanation of the general and essential grandifoliate habit in this class of plants.

The question has next to be answered whether, for the Angiosperms as a group taken by themselves, the grandifoliate or parvifoliate is the most primitive form of plant. After a full consideration of the facts of morphology, especially those relating to the embryonic history of Angiospermous plants, I arrive at the conclusion that the Monocotyledonous and grandifoliate structure was characteristic of the primitive Angiosperm.

Now, my present object in writing this paper is to show that a comparative study of the vascular tissue of certain orders of Angiosperms strongly supports the position reached as a result of morphological study, viz. that the grandifoliate habit (i. e. a comparatively insignificant stem and large sheathing leaves) in Angiosperms has not been derived by reduction and degeneration from the parvifoliate habit, but is primitive and original. Some would suppose that all geophytes and herbaceous perennials with a subterranean stem have been derived by reduction from tall, subaerial, woody plants, and that many have again acquired the character of these latter. But the facts of embryo and seedling morphology, which should, as all botanists teach, reproduce that of the ancestry, do not support this view. In both classes, Dicotyledons and Monocotyledons, the cotyledons are terminal to the axis and the dominating organ of the plant; they are the *first* organs to appear on the scene, the plumule in the majority of cases arising later and in a lateral position, which, in the case of Dicotyledons, appears terminal, owing to the deep forking of the cotyledon having induced the two resulting lobes to separate widely apart from one another, viz. through an angle of 180°. It may be said that the early development and terminal position of the cotyledons is due to their having become adapted to an absorptive function in the embryo-sac; but there is no evidence for this; and further, if the cotyledons are really lateral and subsidiary to the stem, one would expect the *latter* to become the absorptive organ rather than one of the lateral foliar organs, because of its tendency to appear in advance of the cotyledons. Nor, again, is it at all likely that such highly advanced plants as Dicotyledons should exhibit reduction and degeneration of the embryo; it might be conceivable in parasites and saprophytes, which are degenerate in some of their mature organs, but not in plants which in no part of their mature organization exhibit degeneration. Again, if the single cotyledon has been derived either by fusion of two cotyledons or the abortion of the second, one would expect that, occasionally, Monoco-

tyledonous seedlings would exhibit two cotyledons or a bifurcation of the single one; but as far as I am aware none such have ever been found; whereas in Dicotyledonous seedlings cases in which the two cotyledons have fused together into a single organ are very common, and probably, in my opinion, represent *reversions* to the ancestral condition. And the converse phenomenon of bifurcation of the cotyledons is still commoner, showing that this is the direction in which modification is proceeding, and probably has proceeded in the past in the course of formation of the dicotyledonary character. The above facts and ideas have never been faced by those who hold the opposite view to the one I am here promulgating.

Hence I hold that the presence of two cotyledons in Dicotyledons is merely an illusive appearance. There is only *one* cotyledon, as in Monocotyledons; this has become so deeply bifurcated in the great majority of



TEXT-FIG. 1.—*A*. Shoot of *Polyalthia*, illustrating *sympodial* structure, i.e. each leaf *terminates* a stem segment. *B*. Embryo of Monocotyledon, showing similar sympodial structure of plant (after Celakovsky).

cases as to give the appearance of two distinct leaves. In confirmation of this idea I have recently observed a seedling Wallflower in which one of the cotyledons had bifurcated, the two resulting leaves assuming positions with regard to each other precisely like those assumed by the two cotyledons of a normal seedling. Hence the artificial distinction which has always been drawn between the two so-called 'classes' of Angiosperms has not met with much support from the facts of seedling morphology.

I assume, therefore, that *every stem or shoot is essentially a sympodium in the sense that each leaf in its turn constitutes the termination of the main axis*, the shoot as a whole being continued and built up by means of a succession of *branches*, each arising (alternately right and left) from the base of the terminal leaf. And this is precisely what actually happens in all Monocotyledonous seedlings at the earliest stage or stages.

I feel more and more inclined to hold the view that Angiosperms have developed *directly from an ancestor belonging to the Bryophytic level* and that they have not come from either Gymnosperms, Pteridosperms, or

Ferns; on the other hand, it is quite possible that they have descended from a Fern-like ancestor.

In order to determine the nature of the phylogenetic history, we are accustomed to study that of the ontogeny under the right and proper belief that the latter, as a rule, is a condensed recapitulation of the former. But to apply this method right and left, quite regardless of the nature of the prevailing circumstances, as has been done by some botanists, is surely unwise. I am quite unable to follow the argument used by Professor Jeffrey—that because the stem of the seedling Monocotyledon possesses a cylindrical and not a scattered arrangement of the bundles, that therefore the scattered system, as found in the mature stem, has been derived phylogenetically from a cylindrical system. For, supposing, for the sake of argument, that the scattered system of bundles is phylogenetically the primitive one, this character could not possibly be found in the seedling plant, for the cotyledons and earliest foliage-leaves, and, therefore, *a fortiori*, the stem, are far too rudimentary and limited in spatial tissue-development to be able to exhibit anything of the nature of a ranked or scattered grouping of the vascular bundles; there simply is no room for it; hence the cylindrical arrangement must necessarily and inevitably prevail; but in proportion as the successively-formed leaves and the stem increase in size does the scattered arrangement gradually begin to appear upon the scene. This is an excellent instance of a case where the ontogenetic history proceeds in a direction the precise reverse of that of the phylogenetic history. It is, therefore, to the *adult* plant that we must turn in order to unravel the phylogenetic history of the vascular system.

Assuming that the grandifoliate was the primitive condition of the Angiosperm, and that the vascular structure of the stem was derived directly from that of the leaf—and knowing, as we do, that foliar organs tend to retain a primitive structure for a much longer period than is the case with the stem, owing to the greater modification which the latter, as the common carrier of all organs of the plant, must necessarily undergo—we should expect that in a good many cases, viz. in those in which the leaves have not as yet followed the stem in the process of modification, a key to the primitive structure of the stem would be found in the present structure of the leaves; and this I believe to be in many instances indeed the case. In these cases, e.g. many Umbelliferae, the stem possesses an almost what I may call typical parvifoliate structure, i. e. with bundles arranged in a ring; while that of the leaf is typically grandifoliate, i. e. with scattered bundles. Only in rare cases do we find the converse to be true (e. g. *Anemone rivularis*). In a great many cases both organs are equally and similarly modified.

Now, if we assume, for the sake of argument, the case of a plant undergoing reduction from a parvifoliate to a grandifoliate habit, it is quite

impossible to imagine the stem remaining parvifoliate in its vascular structure while that of the leaf becomes modified in a grandifoliate direction; in other words, that a condition like that found in many Umbelliferae and other plants could by this method be attained; for no conceivable reason can be adduced for such a change. On the other hand, if the plant is developing out of a grandifoliate into a parvifoliate habit, it is quite conceivable that the stem, for the reasons above given, should become modified in the parvifoliate direction, while the leaf retains its primitive grandifoliate structure. It is also conceivable that the grandifoliate stem, owing to certain structural peculiarities, such as the possession of a thick sclerotic zone, which is adequate to resist bending-strains should, on eventual elongation, retain the grandifoliate structure while the leaf has begun to assume that of the parvifoliate plant. The above inferences strongly favour the truth of my present theory.

In such cases as that of the Umbelliferae the stem-structure is not quite typically parvifoliate, for the primitive ranked or scattered arrangement of the bundles can still be traced there, for although all the bundles may be practically at one level, i. e. in a single ring or series, they are of three or four different sizes, the largest in some, but not all, cases slightly projecting into the pith, while the smallest are outermost and often quite rudimentary. This latter character is due to the fact that in the grandifoliate ancestor all the ranks of bundles would be well-developed and serviceable to the large leaf-base, and therefore to the stem; but on the acquirement by the latter of the cylindric arrangement, owing to the elongation of the internodes and the reduction in comparative size of the leaves, the bundle-ranks would become condensed and pressed outwards to form a single rank, and the smallest ones, being the most external, would be the first to be obliterated. This can be the only interpretation of the phenomenon; for it is impossible to conceive of the possibility that such plants are modifying their structure in the opposite and converse direction, viz. towards the grandifoliate condition. This is supported by the following facts: In the Ranunculaceae, plants *with a primitive floral structure*, the grandifoliate habit and vascular structure is pronounced, at any rate in many cases, while the traces of it are universal. There is no evidence, nor is there anywhere any sign, that the Ranunculaceae have been reduced and modified from woody parvifoliate ancestors. The Nymphaeaceae, also with a primitive floral structure, have the same grandifoliate habit and vascular structure which have been *retained* probably owing to their having acquired an aquatic habit. I cannot agree with Henslow's view that the grandifoliate vascular structure is the *result* of an aquatic habit either in the plant itself or in its ancestry, for the general effect of this habit upon the vascular structure of stems, as seen in the cases of *Hippuris*, *Hottonia*, *Ceratophyllum*, *Potamogeton*, &c., is extreme reduction and concentration of the bundles to

form a compact mass at the centre of the stem; Nymphaeaceae have in this sense retained their primitive structure in spite of the aquatic habit, although, had they become terrestrial in habit, and developed erect woody stems, they would almost certainly have lost it. In Berberidaceae, Calycanthaceae, and Magnoliaceae, all of which have undoubtedly a primitive floral structure, the majority of plants are woody shrubs, and hence their primitive vascular structure has become largely obscured or lost, but the large, sheathing leaf-bases or stipular appendages of such plants as *Magnolia* and *Berberis Aquifolium* are an obvious reminiscence of their grandifoliate origin, as is also the ranked arrangement of the bundles in the leaves of some genera. In the grandifoliate Berberidaceae, like *Podophyllum*, the ancient vascular structure is present in a most marked degree. The Compositae and Umbelliferae include a great many plants of grandifoliate habit; but, as their floral structure indicates, they are groups advanced in evolution i.e. *in certain special directions*; hence we find that, unlike the Ranunculaceae, their primitive grandifoliate vascular structure is undergoing a great amount of modification, the scattered system of the bundles, where it occurs, being always more or less of a rudimentary nature and showing indications of extinction.

In some plants, where the grandifoliate vascular structure has vanished from the internodes, it is still preserved at the *nodes* which are always the least modified portions of the shoot.

Another interesting point is this: that in the bundles of the scattered medullary system the xylem always tends to surround the phloem. And this amphivasal bundle is precisely that which is characteristic of Monocotyledonous stems, although there, as in the Dicotyledonous stems I am now referring to, the amphivasal character is very often incomplete, the xylem failing to completely enclose the phloem and thereby exhibiting a V-shaped structure. The amphivasal character of the bundle in these stems is the more complete the more independent the latter is of the vascular ring; the nearer the medullary bundle approaches the ring, the more collateral and also the more regularly-orientated does it become; the bundles constituting the ring merely represent the outermost members of the scattered bundle-system marshalled and arrayed in line for the purpose of forming a cylindrical structure which shall be efficacious in resisting bending-strains in the elongating stem; every transition can, in many instances, be seen between the collaterally-constructed members of the ring and the most central and independent of the amphivasal medullary strands. The presence of a cambium, or (as happens in many cases, as where the 'internal phloem'-group is without xylem) the rudiments of such, is a very important feature of the medullary bundles in these Dicotyledons. It indicates that the latter are descended from ancestors the scattered bundles of whose stems possessed secondary thickening to

a greater or less extent. As the more external of these bundles began to form themselves into a ring, and this latter began to increase the extent of its tissues to an ever-increasing degree by means of secondary thickening, this would tend to induce a degradation and eventual disappearance of the cambium of the medullary bundles and, finally, of the bundles themselves.

RANUNCULACEAE.

The grandifoliate habit is strongly represented in this order. They are mostly plants with a squat vegetative stem bearing large leaves. Some, as *R. Ficaria* and *Eranthis*, are typical geophytes having no aerial, leafy stem, but merely peduncles terminating in a flower; most, however, seem rising out of the squat habit, inasmuch as they produce a rather short, and, often, unbranched leafy stem, which, however, soon produces flowers, as in *Delphinium*. The members of this order bear a close resemblance to Monocotyledons, not only in their vascular anatomy but in their habit and the structure of their flowers. But they appear to be less specialized and reduced than most Monocotyledons of the present day, and are probably in several ways and on the whole *more primitive*. It is on account of the primitive floral structure that I regard the prevailing grandifoliate or sub-grandifoliate habit of these plants as also primitive.

Anemone japonica, Sieb. & Zucc.

I will take this plant as a type from this order of plants retaining the, to my mind, primitive scattered or medullary bundle-system.

Leaf: In the typical part of the petiole, which is more or less cylindric, the bundle-system is radio-symmetrical; it consists of four ranks of bundles, of which those of the two outermost ranks are, many of them, situated at the same tangential level, there being, as so often happens, some irregularity in this respect.

Several of the small outermost bundles are rudimentary, possessing either phloem and a sclerotic arc only, or only the latter, their xylem being quite absent. These same rudimentary bundles occur in Monocotyledonous stems and leaves. The innermost rank is represented by a *single* large bundle occupying a perfectly *central* position. The xylem is very V-shaped, subtending a circular phloem. This, then, is the same structure which prevails unmodified in the aerial stem. In the sheathing base of the petiole this scattered system changes gradually into that of the arc, and this takes place by the bundles on the ventral side passing across and fusing with those on the dorsal side, or assuming a position between them, at the same time revolving on their axes through 180°, while all the small rudimentary bundles and a few of the larger ones gradually die out completely or become more and more rudimentary towards the base of the leaf.

Aerial stem: This must be regarded as the typical and least modified

part of the axis of the plant, and hence that part where the primitive vascular anatomy will be most likely to portray itself. Here we find the Monocotyledon-like, scattered system of bundles well shown. There are 3–4 ranks of bundles, though some irregularity obtains here and there. The largest and earliest-formed bundles are innermost, the bundles diminishing in size and development of their tissues from the centre of the axis outwards. Each bundle possesses a circular primary phloem-group situated in a fork of the xylem. All the bundles are leaf-traces. A pith is present, the diameter of which is about equal to the radial distance from the protoxylem of the innermost bundles to the epidermis. There is no sclerotic zone present in the stem.

The *peduncle* also possesses the Monocotyledonous medullary bundle-system, being a stoutly-developed organ.

If this plant has been derived by reduction from an aërophytic ancestor with an elongated woody stem containing a vascular cylinder with the bundles compactly grouped in one rank, one would think that the elongated leafless peduncle of this species is just the kind of organ in which such a one-ranked vascular cylinder would have been retained, unless one admits the somewhat forced idea that this peduncle has been *redeveloped* out of a more humble organ; but if this latter is the case how is it that the aërophytic or parvifoliate structure has not yet been acquired.

The flowering stem of *A. rivularis*, Buch.-Ham. also shows beautifully the grandifoliate or Monocotyledonous structure.

Small forms like *A. nemorosa L.* and *A. apennina L.* have probably been reduced from larger ones like *A. japonica*. There is anatomical evidence of this; for in the petioles of these species a remnant of the medullary bundle-system occurs in the form of a few small rounded *phloem-groups*. And in the bract-node of the stem of *A. nemorosa* I observed four very small bundles pass into the pith, there assume an inverted orientation (two of them having completely lost their xylem) and again pass outwards into the ring.

Thalictrum flavum L.

This plant affords an excellent illustration of the mode of transition in both leaf and stem-structure from the 'Monocotyledonous' to the 'Dicotyledonous' type.

Leaf: In the upper part of the petiole a complete cylinder of bundles obtains, the latter, although of three distinct sizes or primitive ranks, being all in one (rather sinuous) ring; this is owing to the excessively wide hollow pith (Pl. XXXII, Fig. 8). Lower down, two ventral, rather filamentous wings begin to form, and two smaller bundles pass out of the ring at this point, quite on to the dorsal side of the larger bundles there situated. As the wings enlarge, small bundles, presumably arising from the division of the just-

mentioned outlying ones, pass into them ; by further division and enlargement of the strands at the corners of the cylinder the wings become well supplied with bundles, all orientated as those on the dorsal side of the cylinder, while all the time the original cylinder of the petiole is maintained, and the pith becomes narrower and narrower. Eventually the pith becomesobliterated, and the ventral bundles of the cylinder pass across and fuse with those of the dorsal side, some of the smallest ones dying out *in situ* ; the same thing occurs with the bundles of the wings, until finally a wide-sheathing base, enclosing the stem at the node, is formed. There is nothing special to narrate about the mode of union of the petiolar bundles with the stem-cylinder.

Aerial stem: In spite of the wide hollow pith, the scattered arrangement of the bundles prevails here in marked form in the comparatively narrow zone allotted to the vascular tissue, the three ranks of bundles being very independent as regards their relative positions. The xylem is very V-shaped and the phloem pronouncedly circular. A well-marked sclerotic zone surrounds the cylinder (Pl. XXXII, Figs. 9–21).

I will consider two more plants before leaving the Ranunculaceae, viz. *Helleborus* and *Clematis*, as they show some points of interest.

Helleborus odorus, Waldst. & Kit.

Leaf: The ordinary horseshoe-shaped arc prevails in the petiole with a narrow gap on the ventral side. In the sub-divisions of the lamina, however, the inverted ventral bundles occur in various positions, and are fairly numerous ; some very small ones lie closely contiguous to the ventral face of an arc-bundle (Fig. 7). Another one lies rather obliquely on the ventral side and some distance from the arc. I regard them as representing a vestige of the primitive cylinder.

H. viridis, L.

Leaf: The arc of bundles here is almost closed, being very circular, the narrow gap being caused by the ventral groove of the petiole. About the middle of the organ occurs, at one end of the arc, in the case of one leaf examined, a minute bundle with inverted orientation, which lies in the lateral corner of the *ventral* side, and evidently represents the last vestige of the row of inverted bundles which once connected the two ends of the arc ; it dies out below ; its upward course I did not trace. The bundle at the other end of the arc is incurved, but has not gone the length of abstricting off a bundle. A ventral inverted bundle also occurs opposite a bundle near the middle of the arc in the base of one of the subdivisions of the lamina ; further along the same subdivision it does not occur. About half-way down the petiole, in one leaf examined, the two end-bundles of the arc are perfectly *concentric*, with central xylem (Fig. 6); which is due

to the fact that each has abstricted off a bundle on to the ventral side in an imperfect manner, so that the two have remained in contact; or, stating the matter conversely, the sole remaining bundles of the *ventral* part of the cylinder have imperfectly assimilated themselves with the dorsal portion of the latter, each bundle having fused with an end-bundle of the dorsal arc without revolving on its axis; hence the fusion is *with the entire inner face* of the dorsal bundle, instead of, as is usually the case, with one side of it only. So that each concentric bundle here represents a kind of little remnant of the primitively complete cylinder. It is precisely the same phenomenon as we shall presently find reappearing in the case of the concentric bundles of *Paeonia*.

H. niger, L.

Leaf: A perfectly horseshoe-shaped arc here obtains, the end-bundles of which come to lie quite on the ventral side of the petiole and are hence inverted; it is interesting to note that these tend to become obliterated, being very small; and in one case the bundle was quite rudimentary in structure, possessing a few cells of phloem only and no xylem. These facts tend to show that the arc has been derived phylogenetically from a cylinder of bundles.

H. lividus, Ait.

Leaf: The bundles of the petiole are arranged in a wide and very open arc. In passing upwards from the basal region *inverted* bundles appear upon the *ventral* side of the arc; they are mostly smaller than those of the dorsal arc, and lie close to the latter; a bundle of the arc may have two bundles lying obliquely on the ventral side, one at each side of the protoxylem; another bundle is seen with a bundle immediately on its ventral side and perfectly inverse in its orientation. In passing both into the basal region, and also upwards into the lamina, some of these bundles pass in and unite with those of the arc, others appear to die out *in situ* in an oblique position (i.e. at the half-way stage from the purely ventral and inverted state to union with the bundle of the arc); immediately below the lamina the two end-bundles of the arc have each an inverted ventral bundle closely connected with its protoxylem, while the vestiges of another occur further out on the ventral side.

H. foetidus, L.

Leaf: This is anatomically one of the most advanced species, as a very open dorsal arc only prevails throughout the petiole; but traces of the primaeval cylinder occur here and there, as in about the middle region of the petiole a bundle about half the size of most of those of the arc lies at one side of the inner half of the xylem of one of the arc-bundles and is orientated sideways, and is obviously in a position representing a half-way stage on its transit to the ventral side of the arc. Again, in the lamina,

both at the base of the main blade and in the stalks of one or two of its subdivisions, a very small inverted bundle occurs on the ventral side of one of the arc-bundles. By means of these phenomena we are able to see the relationship between the leaf-structure of this species and of those which possess a cylinder.

Clematis Vitalba, L.

Leaf: In the typical part of the petiole a complete cylinder of bundles obtains.

Clematis heracleifolia, DC.

Leaf: In the typical region of the petiole is a wide arc of 12–13 bundles. On the ventral side, and some little distance away, are about four very small *inverted* bundles (Fig. 14). In passing either upwards to the lamina or downwards to the leaf-base, two of these bundles fuse together to form a strand, which then, revolving on its axis, unites with a bundle of the dorsal arc; the two other ventral bundles die out *in situ*.

C. recta, L.

Leaf: Extending throughout the petiole is the following structure: a dorsal arc of about eight bundles, varying in size. The petiole, as also in the last species, has a deep groove on the ventral face. On either side of this groove are, respectively, one and two minute *inverted* bundles, evidently on the point, phylogenetically speaking, of dying out altogether. Before the dorsal arc unites with the stem-cylinder, these ventral bundles, which extend almost to the very base of the leaf, disappear, but I did not determine whether they die out *in situ* or unite with the dorsal bundles.

C. apiifolia, DC.

Leaf: In the typical part of the petiole is a complete, almost circular cylinder of bundles; lower down a flattening occurs on its ventral side, and the bundles thereof begin to pass across and unite with those of the dorsal side; one or two of the more lateral ones persist to the extreme base of the petiole before finally uniting with their dorsal neighbours immediately before the latter unite with the stem-cylinder. By eventual lateral fusion the latter receives three main bundles from the leaf: a median and two lateral ones.

C. alpina, Mill.

Leaf: This is an advanced type from the point of view of its anatomy, inasmuch as the primitive petiolar cylinder has become practically extinct, so that in both the uppermost and lowermost region merely a dorsal arc of bundles obtains; in an intermediate region, however, traces of the ancestral cylinder can be, at least in some leaves, discerned in the form of a minute *ventral* bundle with inverted orientation lying near one of the end-bundles of the arc from which it had presumably become detached (Fig. 15).

In view of the presence of this ventral bundle, this species cannot be said to be so distinct in its petiolar structure from other species, as some authors, e.g. Plitt, has maintained. The petiolar structure of *C. heracleifolia* and *C. recta* is clearly transitional in character between the more primitive type of *C. apiifolia* and *C. Vitalba*, in which, in the typical part of the organ, a complete normal cylinder obtains whose bundles are all equally well developed, and the more advanced type of *C. alpina*, in which nearly, but not quite, all vestige of the bundles constituting the ventral part of the cylinder has disappeared.

We have thus briefly studied some types from the Ranunculaceae, but will postpone a discussion of the results until after the study of types from the other orders of the group has been accomplished.

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PAEONIACEAE.

For the reasons which have induced me to place *Paeonia*, usually regarded as a member of Ranunculaceae, in a separate, independent order, I refer the reader to my article on the subject in the Journal of Botany. The vascular structure of the leaves and stem is very much more like that of Magnoliaceae than that of Ranunculaceae.

Paeonia, sp. (herbaceous type).

Leaf: the petiole contains a horseshoe-shaped arrangement of the bundles, consisting of a large median bundle, two large lateral ones adjacent to it, and two or three smaller ones at either end of the arc; in the upper part of the organ, owing to the incurving of the bundle-arc, an almost complete cylinder is formed (Figs. 19, 20). In the very base of the petiole, whose tissues are already in intimate union with those of the cortex of the stem, the two (2–4 in some species) large lateral bundles, which by this time have become considerably curved in contour, separate off each a bundle which rotates on its axis, assuming thereby an inverted orientation, and takes up a position on the ventral side of the bundle from which it separates; the whole subsequently, by intimate union of the two mutually-inverted parts, becomes a *concentric* bundle, consisting of external phloem and internal xylem; or the ventral may never actually separate from the dorsal portion, but the *whole strand* becomes incurved and finally concentric. From the smaller bundles of the horseshoe, also, minute bundles,

which become inversely orientated, are separated off (Figs. 21, 22); some of these die out below—a fact, coupled with that of their minute and insignificant size, which proves their *vestigial* ancestral character; others pass in and unite with the stem-cylinder. At about this time also the large median bundle of the petiole unites with the stem-cylinder, followed shortly after by the two which are second in number from each end of the arc. The two large concentric bundles are the last to join the stem-cylinder, for before doing so they pass a considerable distance down the internode, forming the characteristic cortical bundles of this plant.

My interpretation of the above structure is this: that the extreme basal portion of the petiolar bundle-system containing the concentric bundles[1] and the minute inverted bundles alone represent the primitive cylindrical structure. The cylindrical structure occurring in the upper part of the petiole, which is formed by incurving of the ends of the dorsal arc of bundles, I regard as a *secondary* phenomenon. If our knowledge of events was confined to Paeoniaceae alone, we should never be able to recognize in the concentric structures the passage from the primitive cylinder to the dorsal arc of bundles; it is only by comparison with similar structures in the closely allied order Magnoliaceae and the allied genus *Helleborus* (see above) where transitional stages are available for our observation, that we are able to interpret the facts in Paeoniaceae in the same way as I have done in the case of the concentric petiolar bundles of *Helleborus*. It is certainly interesting to find that the cylindrical structure of the upper or *typical* portion of the petiole of *Paeonia* is not strictly homologous with that of the petiole in Ranunculaceae, Magnoliaceae, and Anonaceae, but is secondarily derived. However, another mode of interpreting this structure may be as follows: that the *same* primitive cylindric conformation constituting the extreme base of the petiolar system, viz. in the stem-cortex, after completely disappearing for a time as we pass upwards, the dorsal arc of bundles only existing there, *reasserts* itself in the upper or typical region of the petiole; or that the primitive structure of the upper part of the petiole reasserts itself in the cortex of the stem. The comparatively narrow *free* base of the petiole, in the great majority of plants, seems to show the mechanical necessity of the presence there of a dorsal arc of collateral bundles; in the cortex of the stem, in the region where the leaf-base forms part of the latter, this mechanical necessity does not, probably, arise; hence the partial assertion there of the primitive cylindric structure; the reassertion of this primitive cylindric structure occurs in the *upper* or typical part of the petiole; but it is there brought about by a somewhat different constructive agency than is the case with that part of the system which is in the stem; yet this cannot prevent our regarding the

[1] *Paeonia* shows its alliance with Calycanthaceae in the fact that the *ventral* portion of the primitive cylinder does not occur in the leaf proper but *only in the cortex of the stem* (see *infra*).

two structures as *essentially* two, for physiological purposes, interrupted parts of one and the same primitive and ancestral cylinder.

The leaf-base more than half embraces the stem; this character, together with *the large size of the petiole-bundles as compared with those of the stem*, clearly give the impression of the leaf being the more important of the two organs, viz. the one which is likely to have given character and origin to the other, rather than the reverse.

In the *peduncle, concentric* bundles constitute the only foliar-traces; some of them are large, others are very small; they leave the cylinder as arc-shaped strands, which soon close up and enclose a pith (Fig. 23). They eventually enter the sepals of the flower, each breaking up into a number of collateral bundles. Their interpretation is the same as for the vegetative stem.

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MAGNOLIACEAE.

In spite of the fact that the plants of this order are trees and shrubs, there is considerable evidence, both in the external morphology and the internal vascular anatomy, that the entire stem is built up of a succession of phytons, each segment having primarily terminated in a leaf. The large size of the leaves, the way their petiole-bases, by means of their stipules, completely embrace the stem, point to the fact of the leaf having been the dominant organ of the plant, to which the stem was subservient. This is well brought out, as will be seen below, by the study of the vascular structure of stem and leaf.

In the preservation, in the young stem, of the individuality of the bundles of the cylinder, and in their somewhat irregular size and arrangement, we see traces of the primitive scattered system in the central cylinder. Hence I conclude that the arborescent habit of the plant is *derived* and not primitive.

Magnolia tripetala, L.

I will take this species as typical of the genus.

Leaf: In the midrib of the lamina is a complete cylinder of bundles. About half-way down the petiole five or six of these bundles pass into the pith and remain there, all situated on the dorsal side, until such time as the petiolar strands enter the stem; this structure is probably a reminiscence of the primitive scattered system of the herbaceous ancestor.

Stem: At the junction with the stem these medullary bundles again fuse

with the petiolar cylinder. When this has happened the bundles forming the ventral half of this cylinder begin to pass across and unite with those on the dorsal side; this, however, is not completely effected until some considerable distance down the internode of the stem, and long after all trace of the petiole-attachment has vanished. We note the presence in the cortex of the stem of a bundle-system consisting of two or three ranks of strands (as seen in all species of *Magnolia*), some of which are concentric, others arc-shaped, others again with inverted orientation.

Peduncle: In this organ the modifications have not been so great as those in the vegetative stem; there are clearly three ranks of bundles forming, within a limited area, a scattered system of strands. A difference from what usually obtains in stems is here noted, viz. that the largest bundles, which are the latest incoming leaf-traces, are *outermost*, and the smallest innermost, the bundles of the two sizes alternating with each other. They are all very narrow in the tangential direction. In the cortex occur concentric or partially concentric strands arranged in a single ring; their xylem is internal and the phloem external. In the ground-tissue occur sclerotic groups of cells. Between the above-mentioned cortical bundles and those of the central cylinder, and apparently linking up into one the two otherwise distinct systems, is a ring or rank of arc-shaped collateral bundles whose shape is obviously intermediate between that of the bundles of the cortical system and that of those of the cylinder. These represent the components of the dorsal arc which only at this late stage exhibit the completion of the process of union with the ventral strands of the petiolar cylinder (Fig. 33, *Liriodendron*).

The other species in which I found medullary bundles in the petiole are *M. macrophylla*, Michn., *M. acuminata*, L., *M. grandiflora*, L., *M. conspicua*, Salisb., *M. Campbellii*, Hook. f. et Thoms., and *M. Watsoni*, Hook. f.; in the last named, two or three bundles from the dorsal side of the cylinder seem to make a half-hearted attempt to become medullary, but they scarcely leave the cylinder, although they are situated further towards the ventral side than are the other larger bundles of the cylinder.

In *Talauma Hodgsoni* Hook. f. the medullary bundles, which have a very V-shaped xylem, appear in the swollen petiolar base; they emerge from the cylinder on its dorsal side and re-enter it in the extreme base of the organ on its ventral side. The ventral bundles here fuse up together to form two or three large bundles which, revolving on their axes, enter the stem-cylinder independently.

In *Liriodendron* one bundle of the cylinder of the petiole tends to become medullary; at the extreme base the ventral bundles anastomose with the dorsal, the lateral bundles (about two on each side) remaining free and unchanged. There is no persistent cortical leaf-trace system as in *Magnolia*, the change from leaf- to stem-structure taking place more rapidly.

Drimys Winteri, Forst, and *D. aromatica*, F. Muell.

Leaf: Three large bundles, widely separated, occur in this organ; between two of them, in the case of *D. Winteri*, occurs a tiny bundle and at one end of the arc a minute imperfect bundle which is evidently on the way to extinction. *The presence of these small, dwindling bundles seems to point to a former greater development and extension of the arc* to constitute part of a complete cylinder. The somewhat arched character of the larger bundles also seem to point to a time when they were in the habit of abstricting off bundles on the inner side to constitute a medullary system. The arc unites directly with the stem-cylinder. This genus is closely allied to *Illicium*. These plants have the most modified and advanced floral structure of any in the order; it is interesting, therefore, to find a corresponding and parallel advance in the vascular structure.

D. amplexicaulis, Vieill.

Leaf: In the extreme top of the petiole immediately below the lamina and in the midrib of the latter (the only parts of the leaf I was able to examine, and even that in the form of herbarium-material only) there is a dorsal arc of four or five bundles situated at wide distances apart. There is also a medullary system of three or four bundles which are but little smaller than those of the arc; they are situated at varying depths, but none are very far from the arc; they are also orientated in the same manner as the arc-bundles, although one or two lie rather obliquely. Some way up the midrib the arc-bundles are almost concentric, becoming very much inarched, which is probably due to their possessing the characteristic of abstricting off medullary bundles[1]; in close proximity to one of these on the ventral side were two dorsally-orientated small bundles.

The medullary bundle-system does not occur in the two species above described, either in the petiole or lamina, and I am unable, through lack of material, to say whether it occurs throughout the petiole of this species. It must be regarded as a primitive character and its occurrence in this species is probably an index to what must have been the ancestral character of the entire genus. *D. amplexicaulis* is thus more primitive in the vascular structure of the leaf than *D. aromatica* and *D. Winteri*.

Illicium floridanum, Ellis.

Leaf: A single arc-shaped bundle which unites directly with the stem-cylinder; this may be regarded as the most highly-modified and most

[1] This idea is a purely artificial one; phylogenetically speaking, I hold that precisely the reverse process is taking place, viz. fusion of the medullary with the arc-bundles.

recent of the vascular structures in this order; neither stipules nor sheathing leaf-base occur (Figs. 31 and 32). In the structure of the petiole the Illicieae or Winteraceae section of this order shows a certain resemblance to Calycanthaceae, but owing to the absence of any cortical system of bundles they are much more modified and advanced than this latter group.

I need not here occupy space with a description of the remaining genera, as they exhibit nothing of special interest.

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CALYCANTHACEAE

Stem[1]: As regards the cylinder there is nothing special to narrate, the peculiarity of this stem is the presence of the four 'cauline' cortical bundles with *inverted* orientation of their parts (Fig. 25). Starting from a typical region of the axis and tracing the vascular system *upwards* we find on approaching a node that the four cortical bundles begin to divide up in the tangential direction, the two on one side of the stem (corresponding to the two opposed edges of two distinct leaves) being far ahead in this procedure of the two on the opposite side. A large bundle passes off from the cylinder to form the main strand of the leaf. This then gives off a bundle which joins the dividing cortical bundle. From the latter a bundle is now given off at right angles to form one of the lateral bundles of the leaf. Both cortical bundles on the same side then divide up greatly to form a single continuous lengthy strand which constitutes a horizontal connexion between them. Then a second bundle, smaller than the first one, is given off to the lateral region of the leaf, and at the same time a phloem-strand is seen running up towards the midrib-bundle; this is a strand joining the axillary bud-bundles. Both xylem and phloem of the middle region of the horizontal connexion then die out, with the result that the two cortical bundles of that side remain over for the next internode above.

Immediately subsequent to this the same sequence of events occurs on the *opposite* side of the stem.

[1] The entire description here given is equally true for both genera, viz. *Calycanthus* and *Chimonanthus*, and for all species of either.

From the above it will be seen that each leaf, besides the large midrib-strand, receives four bundles (two on each side) from the cauline inverted strands of the stem.

Essentially the same facts were observed in the case of the seedling plant; it would appear, however, that the cauline bundles are rather late in making their appearance, for in the region where the cotyledonary bundles have left the cylinder, but where the cotyledons themselves are not yet detached as distinct organs, there is no sign of the four cauline bundles. But these latter soon after arise from the cotyledonary traces; or, in other words, they pass downwards from the first pair of foliage-leaves above and unite with these traces.

The facts above detailed are usually interpreted as follows: that each cortical bundle is built up by the *lateral* bundles of one-half of each leaf (the main midrib-bundle passing *directly* into the cylinder), which on entering the stem at once unite with a similar leaf-trace descending from the node above. These 'leaf-traces', then, never unite with the stem-cylinder at all, but constitute an independent cortical system. As was the case also with Lignier and Van Tieghem, I was unable to discover any nodal connexion between the cortical bundle and the stem-cylinder, such as Woronin describes; this connexion is effected solely by the midrib-bundles.

Baillon describes a *Chimonanthus*-stem whose leaves (except those, as in the case of some shoots, at the base, which were opposite) were arranged on the shoot according to a $\frac{2}{5}$-spiral, while, in accordance herewith, there were five cortical bundles; this may be regarded as of the nature of a reversion to the primitive condition, for it is interesting to note that the *peduncle* of both genera, which bears *spirally*-arranged foliar organs, has from six to eight cortical bundles which are in five groups (i. e. at two points a pair of bundles occurs). The probable physiological explanation of the presence of these persistent and independent 'leaf-traces' which I would advance is that they serve to give rigidity at the four necessary points to the angles of the quadrangular stem, and have been preserved for this purpose; the same phenomenon of cortical strands occurring in such a position is seen in the square stems of Labiatae, Melastomaceae, and other plants.

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ANONACEAE

The vascular structure of the plants of this order shows clear relationships with that of the Magnoliaceae and Paeoniaceae.

Artabotrys odoratissimus, R. Br.

Stem: Three leaf-trace bundles leave the central cylinder, but not all simultaneously; one of the laterals first passes out and then the other one; finally the median bundle; one of the two laterals (the first to leave the cylinder) is at first three parts *concentric* in structure and later becomes wholly so; the other lateral does not become completely concentric. The median bundle splits into three.

Leaf: As these bundles pass into the leaf the two laterals open out, become quite collateral, and fuse with the two bundles which are the result of splitting of the median bundle. Hence once again three bundles. These, in the upper part of the petiole are three parts concentric, one of the two laterals having hard and soft bast along part of its ventral side. In the lamina the three are fused together into a 3-lobed mass with a sclerotic sheath surrounding the whole, which is perhaps, along with the ventral phloem just mentioned, a reminiscence of a primitive cylinder of bundles possessed by the leaf; while the concentric or sub-concentric bundles in the base of the petiole and stem-cortex probably represent a second partial attempt at reversion to the cylindric structure.

Polyalthia suberosa, Benth. and Hook.

Leaf: In the upper part of the lamina is a very much flattened, undulated *cylinder* of bundles in which is a gap at each lateral angle; the dorsal segments of this cylinder show a tendency to round off into individual concentric strands; in the lower part, near the top of the petiole, the midrib cylinder splits into three parts, of which the middle bundle is quite

concentric, the two lateral horseshoe-shaped, one of which still retains phloem on the ventral side and the other one phloem on part of that side only ; both of them still retain the bast-fibres on that side.

In the upper part of the petiole one lateral exhibits concentric structure with a small inverted ventral segment detached, as if about to pass away from the main strand ; the other lateral and the median bundle have a gap on the ventral side ; the latter bundle has a small strand belonging to the gap some way out on the ventral side ; lower down is an arc of about five collateral bundles.

Stem: By subsequent fusion three bundles are eventually left which unite with the stem-cylinder, the large median one doing so first and then the two laterals (Figs. 34–37).

Eupomatia Bennettii, F. Muell.

Leaf: There is an arc of several (about seven) collateral bundles throughout the organ.

Monodora Myristica, Dun.

Leaf: There is an arc of collateral bundles throughout ; in the upper part of the petiole are three large bundles, the two lateral of which tend to round off somewhat. These arc-shaped bundles (as in the case of all such leaf-bundles wherever they may occur) are due to fusion between the *lateral* bundles of the *dorsal* side and a bundle or bundles passing across from the *ventral* side, these latter being a vestige of the ventral part of a primitive cylinder. (Cf. the concentric and sub-concentric bundles of *Artabotrys*, *Polyalthia*, *Paeonia*, *Berberidopsis*, &c., all of which represent identical structures and have the same explanation.)

Uvaria zeylanica, L.

Leaf: In the *lamina* is a complete cylinder in which the bundles are not individualized ; it is much flattened on the ventral, and convex on the dorsal side (Fig. 38) ; in the upper part of the *petiole* a small gap begins to appear in the middle region of the ventral side, which is barely bridged, i. e. phloem only occurs in that place ; a similar gap appears on one side of the cylinder near the angle between the dorsal and ventral parts. Passing downwards both these gaps become accentuated ; in the second gap above mentioned a small bundle then arises *de novo* ; shortly afterwards connexions take place between the ventral part of the future lateral bundle of that side and the small bundle in the gap ; then the former splits into two, of which one part fuses with the small bundle which arose in the gap ; and then these two, along the part of the cylinder at the angle, go to form the lateral arc-shaped bundle, while the other part fuses with the arm of that side of the large dorsal part of the cylinder. The same thing

occurs on the other side of the cylinder, but not quite contemporaneously. At the base of the petiole are three bundles arranged in an arc and widely separated—a transversely elongated convex median and two arc-shaped lateral bundles.

Cananga odorata, Hook f.

Leaf: In the petiole and lamina of this plant (of which I had only herbarium-material at my disposal) medullary bundles (3-4 in number) occur; they are approximated to the dorsal arc, as in *Magnolia*, are orientated in the same way as the bundles of the arc, and do not occur far away from it; they have a distinct tendency to assume a concentric structure, with central phloem (Fig. 40). This is the only case I observed of *medullary* bundles in the leaf of Anonaceae, and is, therefore, extremely interesting and important. Guillard observed the same phenomenon and it was his note which suggested my own investigation.

In the *peduncle* of *Uvaria Narum*, DC.[1], the bundles of the cylinder are irregular in position and size, each with a large sclerotic dorsal cap. The bundles are arc-shaped. The pith is full of minute, rudimentary bundles, each consisting only of a circular phloem and no xylem; in one place near the cylinder is a transitional form between these medullary bundles and those of the cylinder in the form of a bundle having its phloem partially enclosed by xylem; another small bundle, with a very small group of vessels, was approximated to, and lying at an angle with, a bundle of the cylinder (Fig. 39). The above facts seem to show that the medullary system of bundles is an ancestral or vestigial character which, for some reason or other, has persisted in the peduncle while having vanished from the stem; it is probably to be regarded as a relic of the monocotyledonous scattered arrangement of the bundles. In a peduncle of this species from the Kew Herbarium I could, however, find no trace of the medullary bundle-system; this character may, therefore, vary with the individual; neither does it occur in the peduncles of *U. excelsa* or *U. Kirkii*.

In the *peduncle* of *Melodorum verrucosum*, Hook. f., the bundles of the cylinder are few, large, and horseshoe-shaped. A few (two or three) *medullary* bundles occur; they are small, obscure, and of diverse orientation; occasionally one appears *at the end of one of the incurved limbs of a large bundle of the cylinder*. This fact throws light on the reason for the arc- or horseshoe-shaped contour of the bundles of the cylinder in this plant and also in the genus *Uvaria*; it probably arises owing to constant union, either in the past or present, between the bundles of the cylinder and those of the pith.

The structure of the leaf in this order shows distinct affinities both with

[1] A specimen of which was obtained from Kew Museum, No. 1.

that of the leaf of *Paeonia* and of *Magnoliaceae*; with the former through the two distinct traces of the primitive cylinder in the lower and upper parts of the petiole respectively. In some genera the same formation of concentric bundles occurs, as in *Paeonia* and *Berberidopsis*, and these may almost certainly be interpreted in the same way as in those genera. The presence of what are undoubted representatives of medullary bundles in the leaf of *Cananga* are a perfect replica of the structure in the leaf of some species of *Magnolia*; as in the latter, the vascular structure of the leaf of *Cananga* has taken a half-way stride, as it were, towards ceasing to repeat the phylogenetic character of genuine typical medullary bundles, i. e. at each evolutionary stage the medullary bundles are being set back more and more from the pith into the rank of dorsal arc-bundles, and in the structure of the present-day *Cananga*, as in the Magnolias, we see a transitional stage represented. The other primitive character, viz. the cylindric structure, seems to have disappeared from the leaf of this plant.

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BERBERIDACEAE.

The primitive floral parts of this order find their counterpart in the vascular anatomy which, in my opinion, is also markedly primitive.

I will first consider one of the herbaceous members of the order, viz.:—

Podophyllum peltatum, L.

This is one of the best examples in the vegetable kingdom of a plant-organization in which the leaf appears to be the most important and all-dominating vegetative organ of the plant; in this case, at any rate, the stem is entirely subordinate to the leaf and the vascular structure of the latter clearly gives origin to that of the former.

Fl. stem: Of the 3–4 ranks or rings of bundles here present, the outer one-to-two series are entirely *foliar* and pass out into the two large leaves. The second ring from the centre splits, its bundles each dividing and sending each a branch into the leaf of that side; there are thus left two ranks or irregular rings of bundles, an inner one of large, an outer of smaller bundles which are continuous up into the peduncle, and are doubtless used up in supplying the floral foliar organs (Fig. 41).

Diphylleia cymosa, Michx., has practically the same structure as the last plant; in the peduncle is an external rim of sclerenchyma with the smallest bundles attached to it.

The same is true of *Jeffersonia* and *Leontice*, genera not examined by me.

Epimedium pinnatum, Fisch.

In the *petiole* the scattered arrangement of the bundles of the cylinder is well marked; there are two ranks; the V-shaped character of the xylem is so extreme in some cases that the phloem is almost enclosed by it (Fig. 42).

In the *stem* the ranked arrangement is almost obliterated.

Berberidopsis corallina, Hook. f.

Petiole: In the upper part of this organ there is a complete, transversely elongated cylinder in which no separate bundles can be distinguished. At a lower level one end of the cylinder, followed immediately after by the other, separates and rounds off as a distinct, perfectly *concentric* bundle with external phloem, the xylem on the ventral side of which is much less thick than that on the dorsal side. At the base of the petiole are three bundles: a central horseshoe-shaped, and two lateral concentric ones: these latter again become collateral in the cortex of the stem; and all three unite directly with the central cylinder of the latter (Figs. 45–47).

Akebia lobata, Dcne.

Petiole: The numerous bundles form a complete cylinder; at the base of the organ the ventral bundles pass across and fuse with the dorsal ones; eventually three greatly arched, subconcentric bundles unite with the cylinder.

Nandina domestica, Thunb.

Petiole: The bundles are pronouncedly V-shaped, are in three sizes and more or less scattered, the largest lying innermost; the organ is large and cylindrical (Fig. 43).

Stem: The bundles are much less scattered than in the petiole; a comparison between the two organs in this respect is interesting (Fig. 44).

As regards the other plants of this order, their vascular structure has become much modified away from the primitive type, although the latter can be traced everywhere. For instance, in *Berberis Darwinii* Hook. the primitive vascular cylinder of the petiole has become reduced to an *arc*; in the petiole of *Epimedium alpinum*, L., the ranked arrangement of the bundles is much less distinct than is the case with the species above described, although the V-shaped character of the xylem is quite as pronounced.

Berberis Aquifolium, Pursh.

Leaf: The cylindric petiole has a complete ring of nine bundles which becomes flattened on the ventral side lower down where the organ is marginally winged; as the sheathing base is formed the ventral bundles

pass across and fuse with those on the dorsal side, the leaf-base almost completely encloses the stem, and its median bundle, which is three or four times the size of the others, is the first to join in the stem-cylinder (Figs. 49–52).

Hydrastis canadensis, L.

This plant appears to me more allied to Berberidaceae than to Ranunculaceae, hence I prefer to include it here. The morphological and anatomical structure is exceedingly like that of *Podophyllum*. In the flowering stem is the same scattered monocotyledonous vascular system consisting of three ranks of bundles, which tend to become confused here and there; some of the bundles of the middle rank are larger than those of the inner rank. The same structure occurs in both *peduncle* and *petiole* (Fig. 48).

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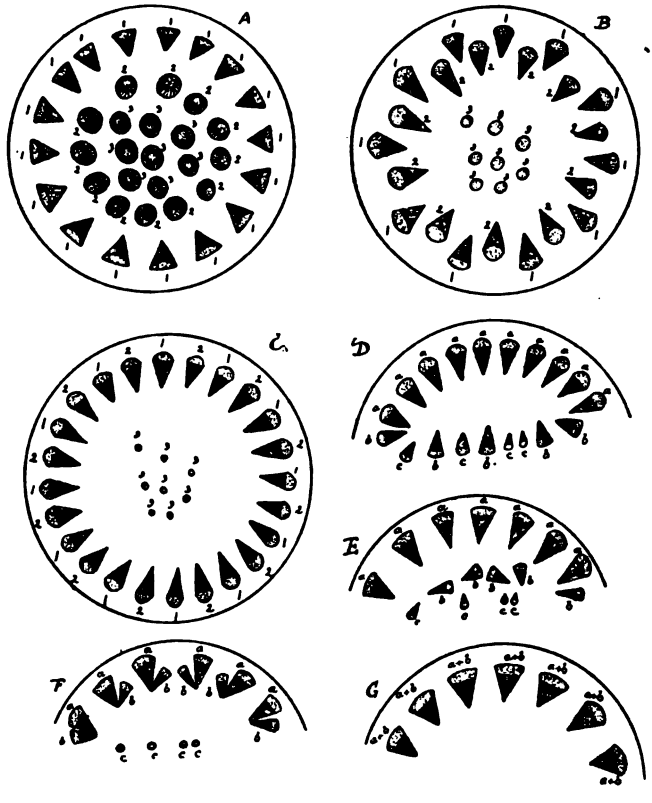
DISCUSSION ON THE VASCULAR STRUCTURE OF THE ABOVE ORDERS.

A comparative study of the essential and general features of the vascular structure of these orders has, for my own mind, at any rate, thrown no small amount of light, not only on the vascular structure of the group as a whole, but also on certain peculiar and characteristic features of some of the orders of which no explanation has hitherto been forthcoming. And yet these characters are surely of sufficient interest and peculiarity to warrant the awakening of some curiosity as to their meaning and origin. It is high time we ceased to be content with the mere observation and recording of structures, and endeavoured to probe into their essential nature and relationships. The first point which accrues as a result of my study of these orders is that the primitive and original vascular system of the leaf and, consequently, that of the stem as (on the phyton theory which I uphold) a product of that of the leaf, is the *complete cylinder* of collateral bundles enclosing a *medullary* system of strands primitively amphivasal in structure. This structure we find preserved in its pristine purity[1] in certain Ranunculaceae and Berberidaceae, plants which are also primitive as regards the structure of their floral organs. In the Ranunculaceae we can trace the steps by which this original structure has become modified, both in the stem and leaf. The scattered or medullary system disappears first

[1] But the *amphivasal* bundle-structure has for the most part been lost.

of all from the stem and is replaced by a hollow cylinder of bundles arranged in a single ring or rank; later on this also vanishes from the leaf leaving a similar hollow cylinder, which in its turn may become reduced to a mere dorsal arc of bundles. In the other orders the medullary system of bundles has largely disappeared; yet we see most evident relics of it in the peduncles of certain Anonaceae and distinct traces yet remaining in the petioles of some Magnoliaceae and Berberidaceae. In these two latter orders we are able beautifully to trace the steps by which the complete cylinder of the petiole has degenerated into a dorsal arc of strands, in some genera all signs of the former having completely disappeared (Text-fig. 2).



TEXT-FIG. 2.—Schematic representation of the stages in the evolution of the *petiolar* vascular structure in Dicotyledons. *A–C* will also apply to the *stem*. *A* is not quite the earliest stage. Numerous variants of this typical general scheme occur.

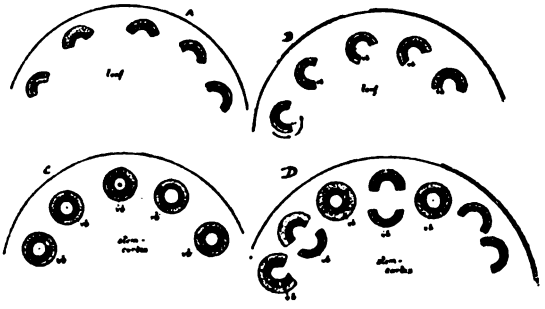
The undeveloped, *imperfect structure* of the medullary bundle system, as also, so often, of the *ventral* portion of the petiole-cylinder, as compared respectively with that of the vascular ring and the dorsal part of the petiole-cylinder, is an index to their *vestigial*[1] character; and this is the

[1] If the ventral portion of the cylinder were not an ancestral character, due to the essentially

proof, or at any rate, a large part of the proof, which is needed to show that the medullary or scattered system, along with that of the complete vascular cylinder, represents for both leaf and stem the primitive and original structure from which all others have been derived. In the Calycanthaceae the dorsal arc of bundles in the petiole is normal and constant for the order, and hence this group may be said to be the most modified and recent of all as regards its actual petiolar vascular structure; this, however, has not been followed by a corresponding advance in the floral structure, which remains one of the most primitive. Yet I shall presently show reason to believe, from a study of the real nature of the cortical bundles of the stem, that the real and essential petiolar structure is not so primitive as appears at first sight.

As a result of this comparative study, we are also now able to explain the peculiar and characteristic cortical bundles of Magnoliaceae and Paeoniaceae, as well as the famous inverted cortical strands of Calycanthaceae.

The concentric bundles of the two former orders are seen to be merely



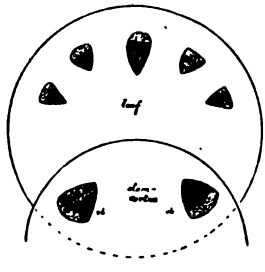
TEXT-FIG. 3.—Schematic representation of part of the petiolar vascular structure of *Paeonia* (*A–C*) and *Magnolia* (*D*), showing phylogenetic origin of the concentric bundles from union of bundles belonging to the dorsal and ventral portions of the primitive cylinder of the leaf. *vb* = bundle of ventral part of cylinder.

the expression of *an imperfect transition from the complete cylinder to a simple dorsal arc of bundles*, for all intermediate stages have been observed between the approach (in the base of the petiole or the cortex of the stem) of the inverted ventral bundles of the cylinder to those on the dorsal side and their imperfect union with the latter to form the concentric strand; complete union, such as occurs in the majority of plants, consists not only of fusion but at the same time of a revolution through 180° on the part of the ventral bundle to form with the dorsal one a single collateral strand, one side only, of course, of each bundle being concerned in this process. In the cortical and petiolar bundles of Magnoliaceae and *Paeonia* and in the petiolar bundles of some Anonaceae and *Berberidopsis* fusion

terminal position of the leaf, and which is gradually being lost, there would be no diminution in size or extinction of the bundles composing it as they gradually approach the base of the leaf.

between the two has occurred but not the complete revolution of the ventral bundle, and union of both sides of each bundle obtains to form the concentric structure as we see it. In the cortex of some Magnoliaceae all transitions towards the completion of this union may be observed—perfectly concentric, three parts concentric, and both normally and inversely-orientated collateral bundles being present (Text-fig. 3). Some of these latter, as is also the case in *Paeonia*, die out in the lower part of the internode without ever taking part in the union with other bundles to form the leaf-traces which unite with the stem-cylinder. In the peduncle of *Magnolia Soulangeana* is an outermost rank of very small and variously, but for the most part, inversely-orientated and *rudimentary* bundles, which doubtless, although I did not trace them, die out below; they had probably emanated from the sepals. Both in Magnoliaceae and *Paeonia* the concentric bundles are *much more typical and in evidence in the peduncle* than in the stem.

After a minute and careful study of the vascular system of the orders now dealt with, I am prepared to affirm without much hesitancy that *the inverted cortical bundles of Calycanthaceae* (allied as this order is to the two others immediately concerned) *are nothing more than the homologues of the inverted bundles above described which occur in the cortex of Paeonia and Magnoliaceae*, and which represent portions of the ventral half of the petiole-cylinder. Instead of, as in the case of Ranunculaceae, Magnoliaceae, and Paeoniaceae, these ventral bundles either uniting with the dorsal strands of the leaf-cylinder or dying out in the internode of the stem, two of them from each leaf [1] persist in an enlarged form as independent cortical bundles in the stem, never passing into the central cylinder. In the cortex of the *peduncle*, however, *concentric* bundles occur, and we may interpret them morphologically as being due to incomplete fusion between the dorsal and ventral bundles of the leaf-cylinder.



TEXT-FIG. 4.—Schematic representation showing the petiolar vascular structure of Calycanthaceae. *vb* = bundle of ventral portion of primitive cylinder.

The proof that the cortical bundles of Calycanthaceae are homologous with the inverted cortical bundles of Magnoliaceae, the meaning and origin of which we know, is afforded by the peduncular structure of both orders, a glance at which is enough to convince us that *this peduncular structure in both orders is homologous* throughout, both as regards the central cylinder and the concentric and other cortical bundles. *As in the case of each of the petiolar concentric bundles of Paeonia*, each cortical bundle in Calycanthaceae is derived (but *phylogenetically*, not *ontogenetically*, as we shall see directly)

[1] i.e. morphologically speaking.

from the lateral bundles of one side of the petiole; in *each* case, viz. of *Paeonia* and Calycanthaceae, the midrib-bundle passes into the stem-cylinder, while the concentric and inversed collateral bundles respectively constitute a cortical system for a less or greater distance down the stem (Text-fig. 4).

Now it is interesting to note that in the majority of plants, when the transition from a cylinder to an arc of bundles takes place in the base of the petiole, it is the dorsal which may be said to dominate and absorb the ventral bundles with which they unite, leaving the dorsal arc as a result of the process. But in the Calycanthaceae precisely the reverse happens: for *the ventral bundles dominate and absorb, so to speak, the dorsal*, with the result that a ventral and, therefore, necessarily *inverted*, system of bundles is left over which persist right through the entire internode; the large size of each inverted bundle is due to the fact that it *represents the fusion of two ventral bundles*. It may be objected that no ventral bundles which unite with the dorsal actually exist in the leaf-base, as in other orders. This is true: but what I have suggested above represents what I believe has occurred in the *phylogenetic* sequence of events, the probable successive steps of which may be indicated as follows:—

(1) The ventral bundles of the petiole-cylinder pass across and fuse with the dorsal ones, a dorsal arc resulting;

(2) a half-way stage, represented to-day by *Paeonia, Berberidopsis*, &c., in which both ventral and dorsal bundles are equally strong and both refuse, as it were, to effect a complete union;

(3) the dorsal bundles, with exception of the midrib, pass across and unite with the ventral, a ventral arc, complete only in its lateral portion, resulting;

(4) the ventral bundles are pushed further and further downwards until they disappear from the leaf and occur only in the cortex of the stem, as in Calycanthaceae.

Hence, it appears to me to be not strictly, but rather only metaphorically, true to say that the inverted cortical bundles are leaf-traces in the sense that they emanate directly from the leaves. My own observations certainly show that, as regards the ontogenetic history, four lateral bundles from the petiole-arc pass into the cortex of the stem and unite with *strands which are already in existence there as*, in a sense, *cauline* strands. These latter are far too large in size to belong to the lateral bundles of the petiole. My conclusion is, that they belong in present actuality, though not phylogenetically nor essentially, to the stem and not to the leaf. Phylogenetically, they *are* leaf-traces. This is in agreement with the conclusions of Van Tieghem and Hérail; the older authors like De Bary are, I am convinced, in error in regarding these bundles as leaf-traces.[1]

[1] Hérail says in effect that the inverted cortical bundles of Calycanthaceae are not derived from

The facts of the ontogenetic apical development of the stem would probably be of no use to us in settling this question, for the cortical bundles would probably be seen in process of formation above the insertion of the highest and youngest leaf, i.e. they ought to, from what we see in the mature structure; the fact that these strands have no connexion with the central cylinder and only an indirect connexion with the leaf shows them to be *cauline*.

During this sequence the dorsal midrib is early differentiated and remains unaffected by any subsequent events.

The persistence of the *inverted* orientation of these bundles may perhaps be due to two physiological causes: (1) the fact that these bundles bear no relation to the stem-cylinder; (2) the fact that a dense arc of sclerenchyma occupies the dorsal (outer side), the usual position for the bast-fibres, causing the factor of orientation to become an indifferent one.

Hence the structure of the leaf of Calycanthaceae is not so highly modified as would at first appear; the presence of a dorsal arc of bundles *only* is merely apparent: the ventral system exists as well, but has become transferred, as in *Paeonia*, into the cortex of the stem.[1]

Thus we see that the structure of the Calycanthaceous stem is not so peculiar and so isolated as has hitherto been supposed; in the preceding pages I have, it seems to me, fully accounted for its supposed vagary in possessing such peculiar structures as inverted cortical bundles. I have also accounted for and explained the meaning and origin, hitherto unattempted, of the concentric cortical strands of Magnoliaceae and *Paeonia*.[2]

Throughout these various orders we find the different phylogenetic stages stereotyped in the actual structure of present-day species. This is especially well seen in the Anonaceae. In *Artabotrys*, *Polyalthia*, and *Uvaria* we see a primitive structure extending through the greater part of the leaf, the base only showing the more modified, advanced structure; in *Monodora*, the structure which occurs only in the basal region of the leaf of *Uvaria* is here typical for the organ as a whole, indicating a greater advancement for the genus; the *arc-shaped* contour of the lateral bundles in this plant (*Monodora*) represent in the ontogeny a congenital fixation of

the *lateral* bundles of the leaf, but, on the contrary, they supply these bundles to the leaf; they possess a cambium and a much larger amount of phloem than the lateral leaf-bundles; their whole structure is different and more voluminous. Van Tieghem says that these bundles 'sont donc, au même titre que la stèle, des éléments constitutifs de la tige, et c'est par erreur qu'on les a considérées jusqu'ici comme de simples méristèles foliaires.' I have arrived, quite independently of both these authors, at the same conclusion.

[1] This fact need not seem strange when we remember that stem and leaf are so intimately conjoined and related as I hold, on the phyton theory, to be the case.

[2] The structure of the peduncle in all three groups represents the ancestral point of common agreement and union, for this organ has varied less in structure in the course of evolution than has the vegetative stem; hence the structure of the latter represent the diverging lines of disagreement and separation; but we must not be misled ther

that stage in the phylogeny in which a bundle from the ventral side is in the act of uniting with a bundle of the dorsal arc; in other orders, viz. Ranunculaceae and Magnoliaceae, we see this process actually taking place in the ontogeny; in Anonaceae and Paeoniaceae the process is no longer seen in actual operation, but certain stages of it have become fixed and arise congenitally as such in the individual life-history. *Eupomatia* represents the most advanced type of this order in which an ordinary arc of bundles extends throughout the leaf. The concentric bundles which occur in the leaf of this order are homologous with those which occur in the cortex of the stem of Magnoliaceae and *Paeonia* which is only another instance of the fact that a given structure which in one group of plants is found only in the leaf, may in another occupy that part of the stem which is a direct continuation of the leaf; this supports my view of the origin of the cortical bundles of Calycanthaceae and also the phyton theory.

In conclusion I must thank the authorities of the Royal Gardens, Kew, for valuable material supplied to me for this work.

EXPLANATION OF FIGURES IN PLATES XXXII AND XXXIII

Illustrating Mr. Worsdell's paper on the Vascular System in the Ranales.

Abbreviations used:—*v.* *cb* = ventral cortical bundle; *vb* = ventral bundle; *cx* = cortex; *mb* = medullary bundles; *cc* = central cylinder of stem; *ax. c* = cylinder of axillary branch; *lb* = leaf-base; *cb* = cortical bundle; *st. b* = stipular bundles; *hp* = hollow pith; *p* = pith.

All figures are diagrammatic, and represent transverse sections.

Fig. 1. *Anemone rivularis*, Buch.-Ham.; flowering stem.

Fig. 2. *Anemone nemorosa*, L.; segment of central cylinder from bracteal node of flowering stem.

Fig. 3. *Anemone nemorosa*, L.; typical region of petiole.

Fig. 4. *Anemone apennina*, L.; typical region of petiole.

Fig. 5. *Anemone sylvestris*, L.; flowering stem.

Fig. 6. *Helleborus viridis*, L.; typical region of petiole.

Fig. 7. *Helleborus odorus*, Waldst. and Kit.; subdivision of lamina.

Fig. 8. *Thalictrum flavum*, L.; typical region of petiole.

Figs. 9, 10, 11. *Thalictrum flavum*, L.; successive modifications in sheathing base of petiole in passing from above downwards.

Fig. 12. *Thalictrum flavum*, L.; segment of flowering stem.

Fig. 13. *Clematis Vitalba*, L.; stem.

Fig. 14. *Clematis heracleifolia*, DC.; typical region of petiole.

Fig. 15. *Clematis alpina*, Mill; typical region of petiole.

Fig. 16. *Caltha palustris*, L.; typical region of petiole.

Fig. 17. *Caltha palustris*, L.; lower region of petiole.

Fig. 18. *Caltha palustris*, L.; base of petiole after union with stem.

Fig. 19. *Paeonia*, sp. (herbaceous type); typical region of petiole.

Fig. 20. *Paeonia*, sp. (herbaceous type); base of petiole.

Figs. 21, 22. *Paeonia*, sp. (herbaceous type); successive stages in union of leaf-base with stem.

Fig. 23. *Paeonia*, sp. (herbaceous type); peduncle.

Fig. 24. *Calycanthus floridus*, L.; typical region of petiole.

Fig. 25. *Calycanthus floridus*, L.; stem.

Fig. 26. *Calycanthus floridus*, L.; peduncle.

Fig. 27. *Manglietia insignis*, Blume (Magnoliaceae); typical region of petiole.

Figs. 28, 29, 30. *Manglietia insignis*, Blume; successive stages in union of petiolar vascular system with that of stem.

Fig. 31. *Illicium Cambodianum*, Hance; typical region of petiole.

Fig. 32. *Illicium Cambodianum*, Hance; union of leaf-base with stem.

Fig. 33. *Liriodendron tulipifera*, L.; peduncle.

Figs. 34–37. *Polyalthia suberosa*, Benth. and Hook; successive stages from typical part of petiole (Fig. 34, monostele or cylinder) downwards through the intermediate 'polystelic' condition (Fig. 35, see *Paeonia*) to the simple arc (Fig. 36), and the union of the latter with the stem-cylinder (Fig. 37).

Fig. 38. *Uvaria zeylanica*, L.; typical region of petiole.

Fig. 39. *Uvaria Narum*, A. D. C.; peduncle.

Fig. 40. *Cananga odorata*, Hook. f.; petiole.

Fig. 41. *Podophyllum peltatum*, L.; flowering stem.

Fig. 42. *Epimedium pinnatum*, Fisch.; typical region of petiole.

Fig. 43. *Nandina domestica*, Thunb.; segment of petiole.

Fig. 44. *Nandina domestica*, Thunb.; segment of stem.

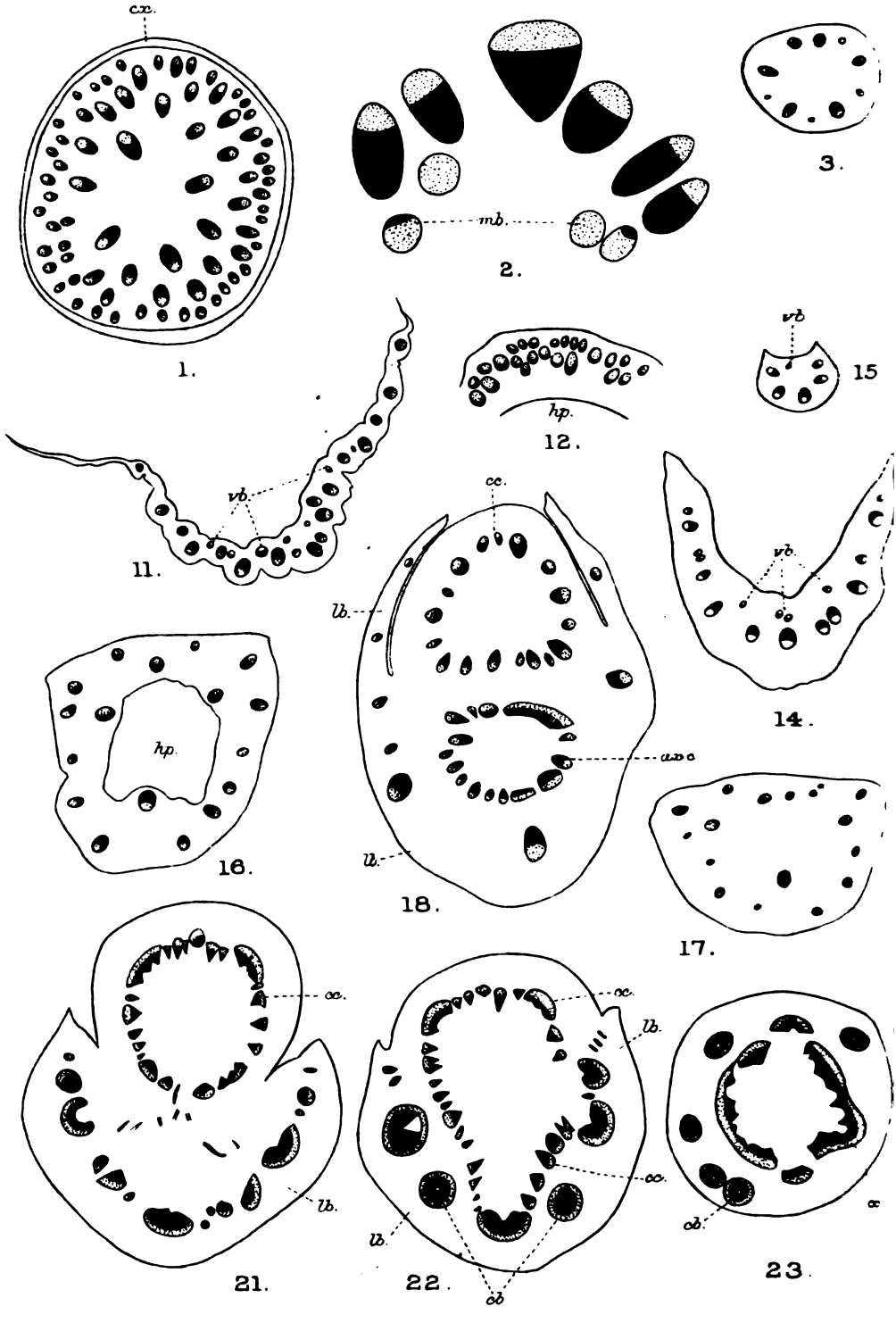
Fig. 45. *Berberidopsis corallina*, Hook f.; typical region of petiole.

Fig. 46. *Berberidopsis corallina*, Hook f.; lower region of petiole.

Fig. 47. *Berberidopsis corallina*, Hook f.; union of leaf-bundles with stem-cylinder.

Fig. 48. *Hydrastis canadensis*, L.; flowering stem.

Figs. 49–52. *Berberis Aquifolium*, Pursh.; successive stages in passage from typical part of petiole (Fig. 49) to union of leaf-base with stem (Fig. 52).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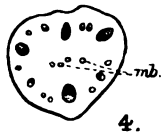

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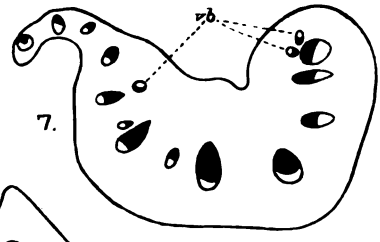

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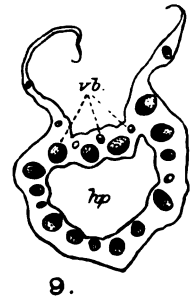

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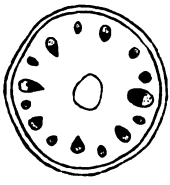

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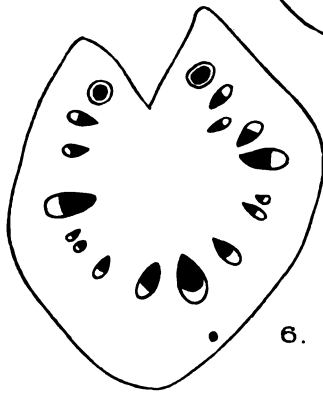

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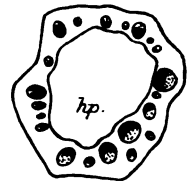

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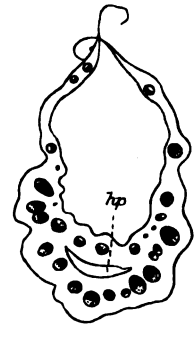

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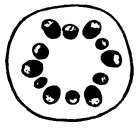

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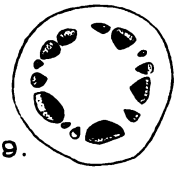

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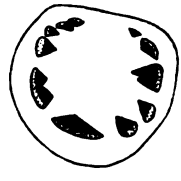

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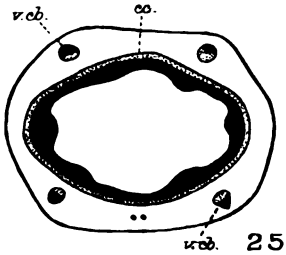

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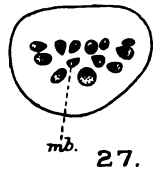

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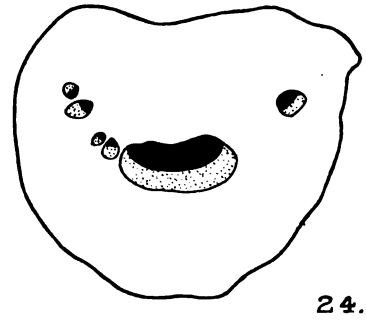

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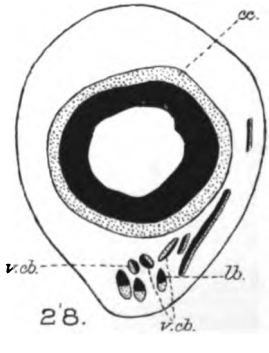

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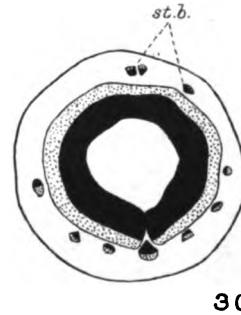

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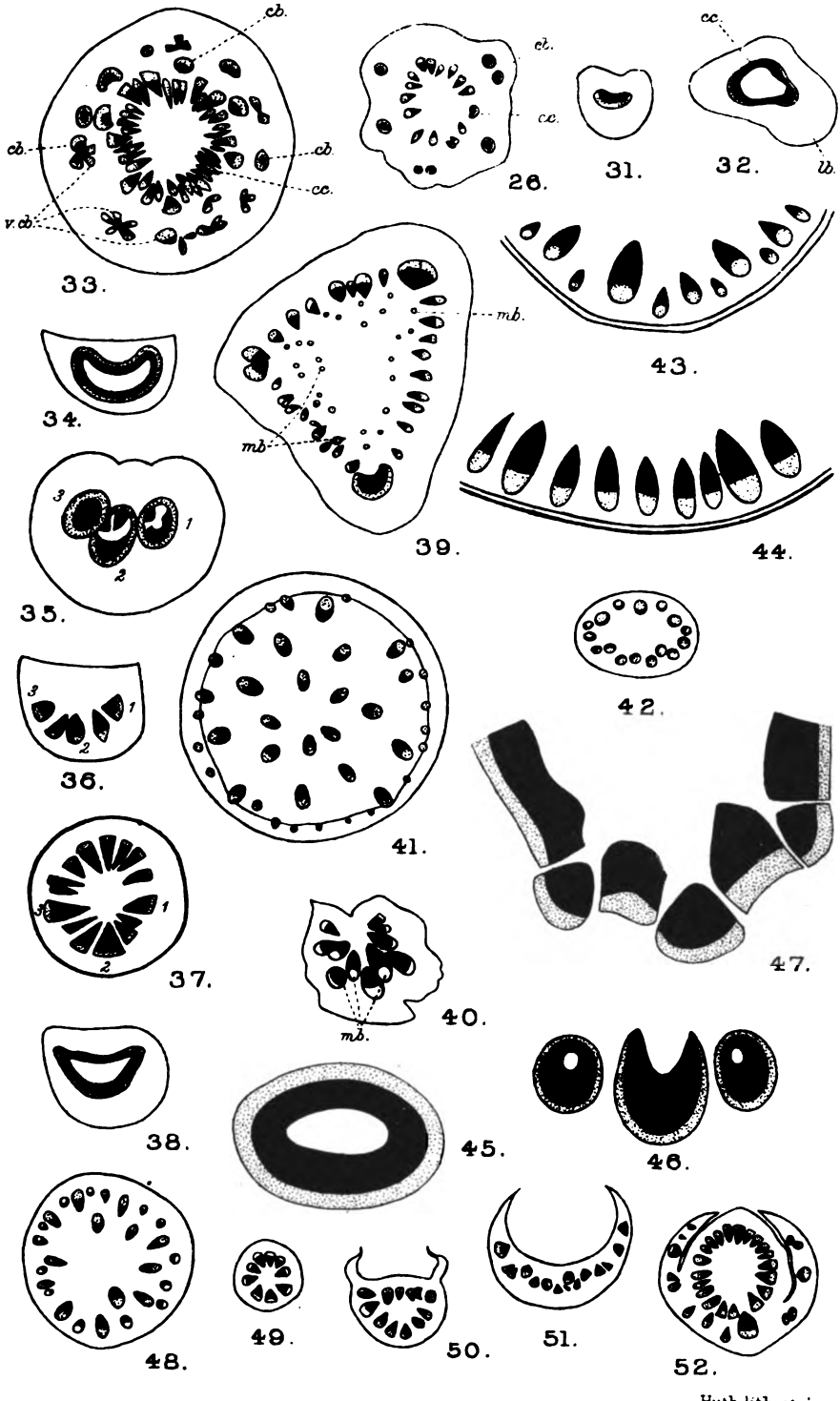
SCULAR SYSTEM.



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WORSDELL – VASCULAR SYSTEM.

On Bensonites fusiformis, sp. nov., a fossil associated with Stauropteris burntislandica, P. Bertrand, and on the sporangia of the latter.

BY

RINA SCOTT, F.L.S.

With Plate XXXIV and seven Figures in the Text.

SEVERAL years ago Miss Benson noticed in her slides from Burntisland (Lower Carboniferous), curious spindle-shaped objects, tapering at one end and at the other terminating in a head, containing sometimes one spherical body and sometimes a double, hourglass-shaped body, Plate XXXIV, fig. 6.

Since then they have been frequently observed, especially in material rich in *Stauropteris burntislandica*, P. Bert. They have been provisionally named *Bensonites fusiformis* by Dr. Scott.

Text-figs. 1, 2, and 3 are photographs of *Bensonites* cut in a longitudinal direction. Just recently it has been discovered that the *Bensonites* is borne on a pedicel. Text-fig. 1, *p*.

We have not been able to find a clear case of attachment of the pedicel to a *Stauropteris burntislandica* petiole, Plate XXXIV, fig. 4, but they are very probably borne in pairs on the lateral pedicels shown in Plate XXXIV, fig. 3. Miss Benson has slides which confirm this.

The 'body' of *Bensonites* appears to be composed of regular spherical cells surrounded by an epidermal layer, which is often imperfectly preserved (it is absent in Text-fig. 1). It is well seen, however, in longitudinal section in Text-fig. 2, where it completely envelops the *Bensonites*, and in transverse section in Text-figs. 4 (*a* and *b*) and 5 (*c*). The transverse section in Text-fig. 5 is strikingly like a section of the gland of *Lyginodendron oldhamium*.[1]

From the first a strand of delicate elements has been noticed running up from the base, Text-figs. 1 and 3 (*v.s.*). Lately we have been fortunate in being able to demonstrate without any doubt that this is really a vascular strand, Plate XXXIV, fig. 5. Text-fig. 6 shows the two spiral elements indicated in Plate XXXIV, fig. 5, enlarged.

[1] Oliver and Scott, on *Lagenostoma Lomaxi*, Phil. Trans., vol. cxvii, 1904, Plate VIII, Fig. 18.

[Annals of Botany, Vol. XXII. No. LXXXVIII. October, 1908.]

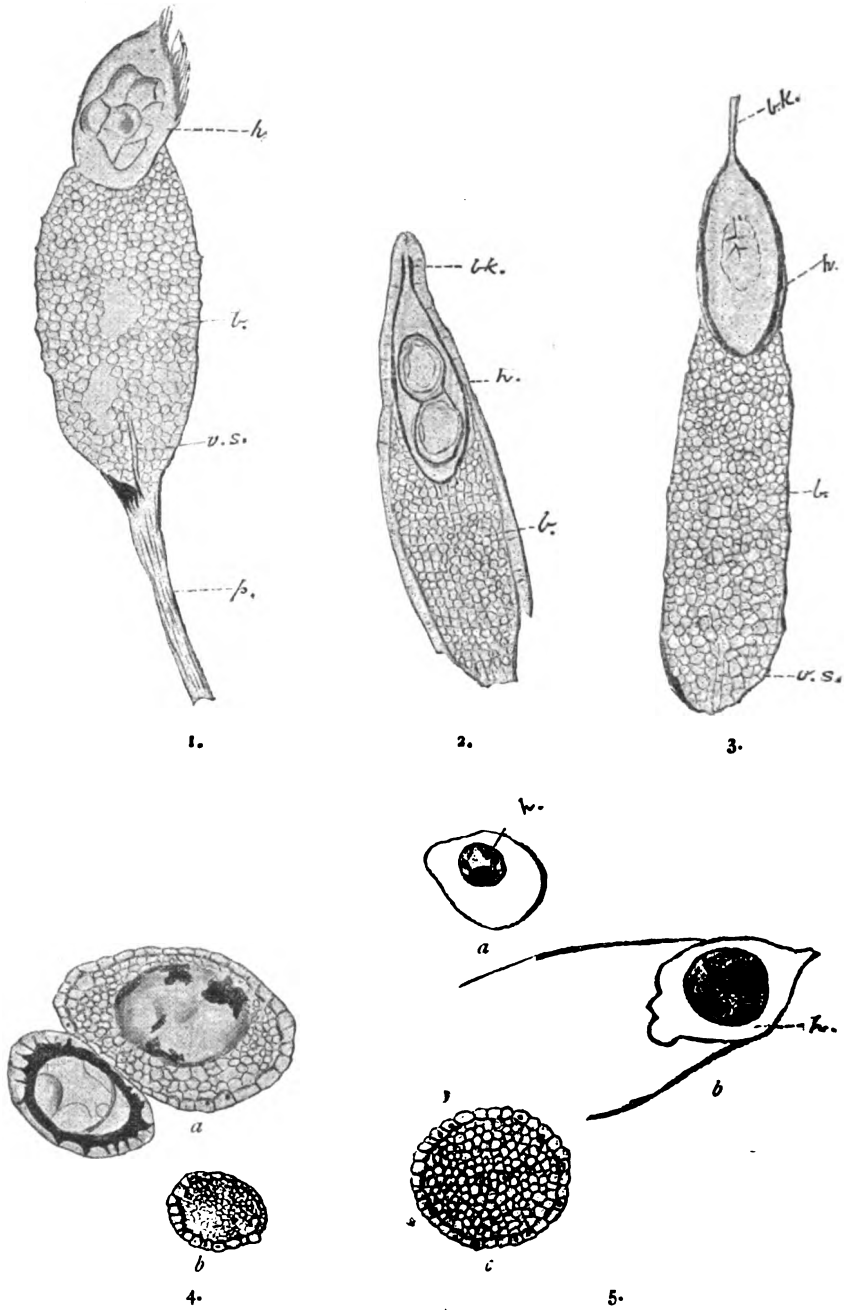


Fig. 1. *Bensonites fusiformis*, showing head *h*, body *b*, pedicel *p*. × 50. S. Coll. 443.

Fig. 2. *Bensonites fusiformis*, showing beak *bk*, head *h*, and body *b*. The epidermal layer is complete. × 50. S. Coll. 383.

Fig. 3. *Bensonites fusiformis*, showing beak *bk*, head *h*, body *b*, and vascular strand *v.s.* shown more magnified in Fig. 6. From a slide of Miss Benson's. × 50.

Fig. 4. *Bensonites fusiformis*, showing (*a*) two adjoining transverse sections, one through the lower part of the head, and one through the middle of the head. The small section (*b*) is through the lower end of the 'body'. S. Coll. 383. × 50.

Fig. 5. *Bensonites fusiformis*, trans. (*a*) and long. sections (*b*) through the head *h*, and (*c*) trans. section through the broadest part of the body. S. Coll. 383. × 50.

The 'head' of the *Bensonites* is well marked off from the 'body' and ends at the apex in a long beak: Text-figs. 2 and 3 (*bk*). A very common form for the contents to assume is that of an hour-glass, Plate XXXIV, fig. 6, Text-figs. 2 and 7, or it may be spherical in shape: Text-fig. 5 (*a* and *b*).

The dimensions of an average *Bensonites* are as follows:

Length, 1·3 mm.; *width*, ·42 mm.

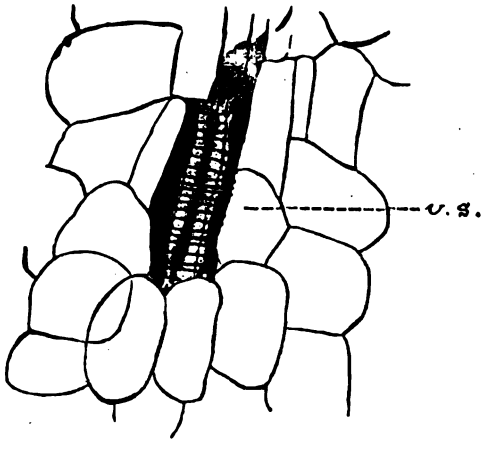
Width of pedicel from ·05—12 mm.

Length of 'head' ·60 mm., the longest observed 'hour-glass' head.

Width of 'head' ·24 mm.

I will now describe the sporangia, which we have recently found associated with *Stauropteris burntislandica*, P. Bert., Plate XXXIV, figs. 1 and 2, and then return to the discussion of the *Bensonites* problem.

The sporangia of another *Stauropteris*—*S. oldhamia*, with their germinating spores—have been fully described by Dr. Scott.[1]



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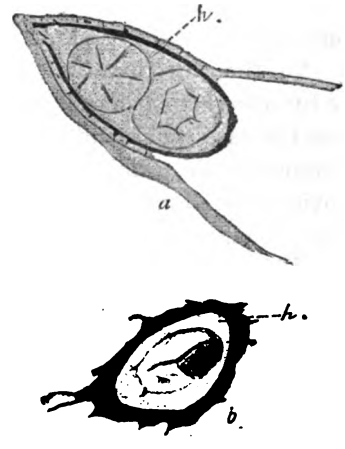

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Fig. 6. *Bensonites fusiformis.* Enlarged drawing of the vascular strand, *v s.*, showing two spiral elements. × about 400.

Fig. 7. *Bensonites fusiformis* (*a*) longitudinal section of 'head' with hour-glass-shaped contents, and (*b*) oblique section of another 'head'.

Stauropteris burntislandica, so common in the Burntisland material, has now disclosed its sporangia, and they will be found to be extraordinarily like those of its younger relation *S. oldhamia*.

They occur in great quantity in the region of the *Stauropteris* petioles, Plate XXXIV, fig. 4. A single sporangium is shown in Plate XXXIV, figs. 1 and 2.

[1] D. H. Scott, Germinating spores in a fossil fern sporangium, New Phytologist, vol. iii, 1904. Sporangia of *Stauropteris oldhamia*, ibid., vol. iv, 1905. The occurrence of germinating spores in *Stauropteris oldhamia*, ibid., vol. v, 1906.

The sporangia are almost spherical in shape; the 'stomium' is well shown in Plate XXXIV, figs. 1 and 2, *st.*

They are generally filled with spores. I have been fortunate in finding one sporangium filled with germinating spores (Plate XXXIV, fig. 2) though the stages of germination are not so clearly shown as in *S. oldhamia.*

This is of the greater interest as it is the third example in this genus in which the spores have been found in a state of germination. It is curious that up to the present this should be the only genus of fossil plants in which this phenomenon has been observed.

We now return to a fuller consideration of the 'head' of *Bensonites*, and to a discussion of the various views which have been held about the fossil since it was first noticed.

The strong resemblance of the sporangial wall of *Stauropteris burntislandica* to the epidermis of *Bensonites* gave rise to the suggestion that the '*Bensonites*' might be an aposporous outgrowth from a sporangium, the 'body' representing the prothallus bearing an archegonium with a beak, containing an embryo (Text-figs. 2 and 3).

On the other hand transverse sections through the head have been sent me by observers as examples of 'new' megaspores (Text-fig. 7, *b*). If this were the case we should have to look on the whole 'Bensonites' as a megasporangium or primitive ovule, and on this view a female organ would be provided for *Stauropteris burntislandica*, the ordinary sporangia becoming microsporangia.

This tempting view, however, is difficult to hold, as in the much better preserved *Stauropteris oldhamia*, in which the structure of the petioles and sporangia is hardly distinguishable from *Stauropteris burntislandica*, there is no sign (although I have searched every available specimen) of any such organ corresponding to *Bensonites.*

The third theory is that *Bensonites* is a gland. The very striking resemblance of the tissue to that found in *Lyginodendron oldhamium* glands supports this view. It is known that glands of some importance occurred in one genus of Carboniferous plants, so that it is quite probable that they should be found in others too.

If this view be the right one, the glands must have been of some considerable importance; they are supplied with a vascular strand and a beak, through which their contents could have been ejected. The 'head' in this case would represent the partially disorganized tissue, in which the secretion was formed.

DESCRIPTION OF PLATE XXXIV

Illustrating Mrs. D. H. Scott's paper on *Bensonites* and *Stauropteris*.

Fig. 1. *Stauropteris burntislandica*, P. Bert. Sporangium containing spores. *st.* = stomium. × 50.

Fig. 2. *Stauropteris burntislandica*, P. Bert. Sporangium containing germinating spores. S. Coll., 436. × 50.

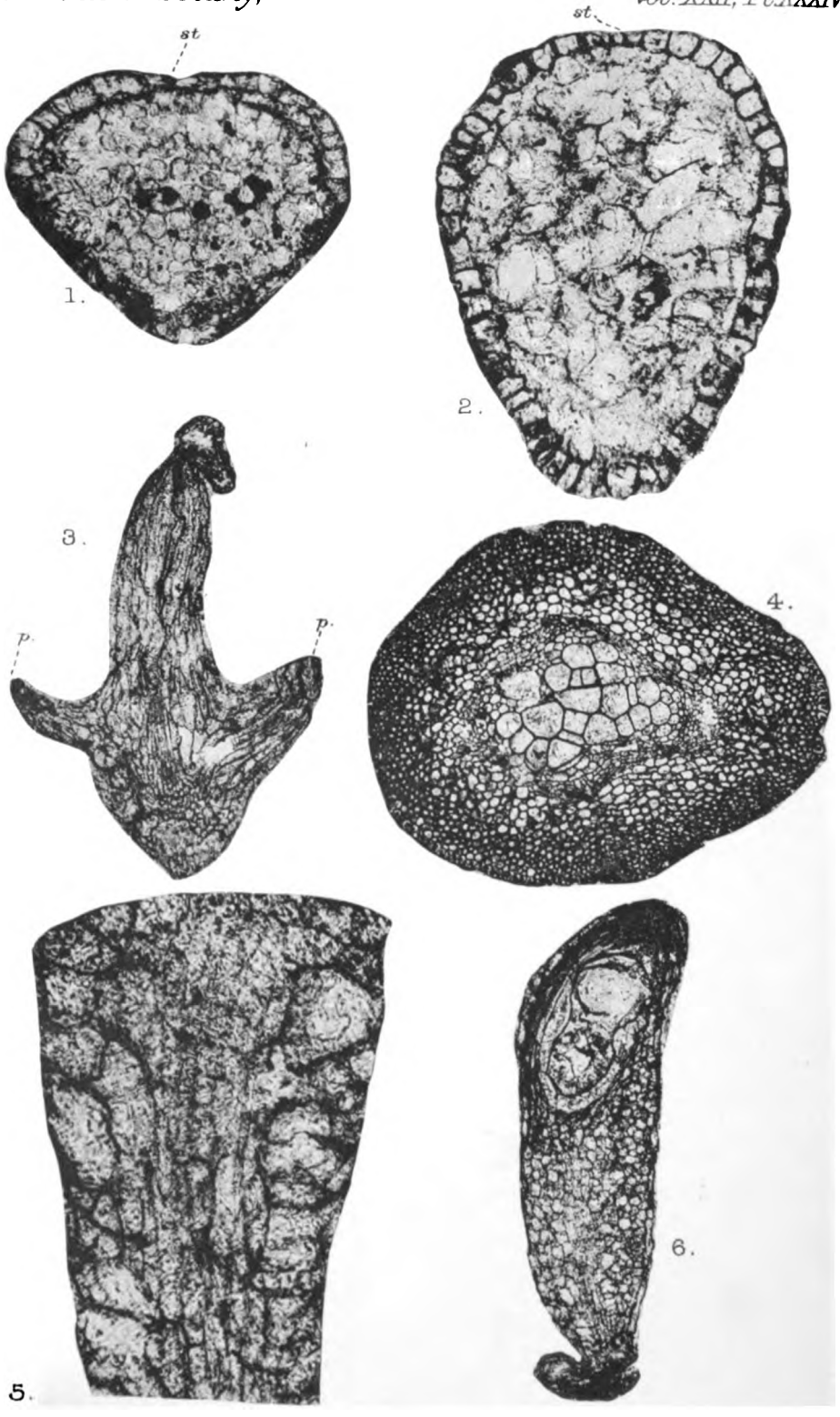
Fig. 3. *Stauropteris burntislandica*, P. Bert. Pedicels, *p*, *p*, probably bearing *Bensonites fusiformis*. × 50.

Fig. 4. *Stauropteris burntislandica*. Trans. petiole. S. Coll. 489. × 50.

Fig. 5. *Bensonites fusiformis*. Longitudinal section through part of the base, showing vascular strand, which is shown in detail in Text-fig. 6. × about 400.

Fig. 6. *Bensonites fusiformis*. Longitudinal section with portion of pedicel and hour-glass-shaped 'head'. × 50.

The photographs are by Mr. Tams, from slides in the Scott collection.



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R. SCOTT — BENSONITES AND STARUOPTERIS.

On the Seedling Structure of Gymnosperms. I.

BY

T. G. HILL, A.R.C.S., F.L.S.,

University of London; University and Goldsmiths' Colleges;

AND

E. DE FRAINE, B.Sc., F.L.S.,

University of London; Goldsmiths' College.

With Plate XXXV and eight Figures in the Text.

THE study of seedlings was commenced by one of us seven years ago, but the work, occupying a secondary place as an investigation, made but slow progress. The appearance of the results obtained by Miss Sargant [1] rendered it desirable to modify the original scheme, and it was decided to ascertain, as far as possible, the precise value of the transition-phenomena in questions relating to the phylogeny of the higher plants. With this end in view, it was considered advisable to examine as large a number of species as possible in well-marked cycles of affinity, and the Gymnosperms, Piperales, Centrospermae, and those Natural Orders of the Tubiflorae more directly related to the Scrophulariaceae were selected as being likely to afford the evidence required.

Whether this somewhat extensive programme will be completed remains to be seen; it is probable that the work on the Scrophulariaceae will not be continued, for, in the light of the knowledge gained of the groups mentioned, together with others, it appears possible that the expenditure of so much time and labour may not be necessary.

The work on the Gymnosperms is fairly well advanced, and it has been decided to publish the results obtained from the study of the Taxaceae, the Cupressineae, and the Taxodiinae of the Abietineae.

As regards methods, little need be said. In nearly all cases the seedlings were microtomed. The staining of the sections presented some initial difficulty; those excellent stains haematoxylin and safranin not only

[1] Sargant, E.: A Theory of the Origin of Monocotyledons, founded on the Structure of their Seedlings (Annals of Botany, xi).

[Annals of Botany, Vol. XXII. No. LXXXVIII. October, 1908.]

require much time, but they also give, for this particular work, a very poor differentiation. The method finally pursued was as follows:—

The sections were stained on the slip for about half an hour in a saturated solution of gentian violet in 50 % alcohol and were then, without washing, transferred to a saturated alcoholic solution of vesuvian brown for a few seconds; the excess of stain was then washed out in a mixture of absolute alcohol and xylol, and the sections rinsed, when necessary, very rapidly in absolute alcohol. They were finally cleared in xylol and mounted in balsam. If the process is properly performed, the lignified tissues are coloured a very bright violet, while the phloem stains brown, and the ordinary parenchyma a light brown.

The number of seedlings of one species prepared varied according to the supply. The usual course was to cut up at least three individuals whenever possible, and more when necessary.

This work would have been impossible except for the kindness of many in supplying material. As we still have hopes of obtaining further contributions, we propose to postpone the expression of our indebtedness until our results are in a more forward state of publication.

Finally, it may be remarked that our general conclusions will not be stated until the whole of the facts and the immediate conclusions derived therefrom have been published.

TAXACEAE.

Cephalotaxus pedunculata, Sieb. & Zucc. On germination the seed is carried up above the level of the soil by the two cotyledons, which remain embedded within the endosperm and function as organs of absorption until the tissues of reserve are depleted, by which time the plumule is well advanced. Ultimately the seed is dropped, and the seed-leaves perform an assimilatory rôle (Figs. 1 and 2, Plate XXXV).

The structure of the cotyledon requires but a brief description. The epidermis is covered by cuticle which is poorly developed, excepting over the guard-cells, where it is very thick. The mesophyll consists of an undifferentiated mass of parenchyma with fairly abundant intercellular spaces. Cells, which appear to be of a secretory nature, with dense and deeply-staining contents, occur within the mesophyll, being situated especially in the hypodermal region of the dorsal surface of the leaf and on the dorsal side of the vascular bundle.

Each cotyledon has a single vascular strand which is somewhat tangentically elongated. Directly abutting on to the soft bast is a mass of rather wide and long cells, fibrous in nature, which, when mature, are devoid of contents, closely packed together, and unlignified.

The xylem exhibits a pronounced mesarch structure; the centripetal

elements, however, are not very numerous. This feature has already been described by Worsdell,[1] and, to save reiteration, it may here be remarked that Worsdell describes the occurrence of mesarch structure in *Taxus, Ginkgo*, and other plants, and the presence of transfusion tracheides in *Cephalotaxus, Taxus, Sequoia gigantea, Widdringtonia Whytei, Libocedrus decurrens*, &c.

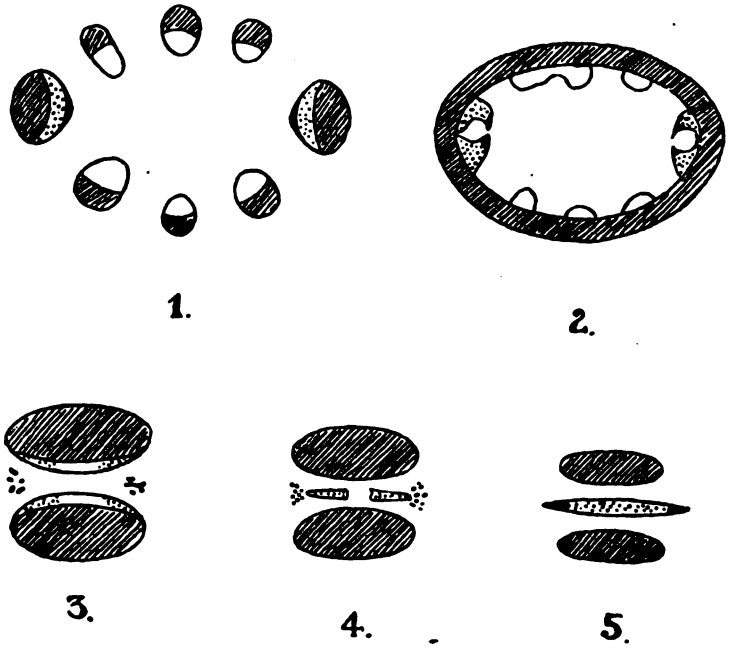


DIAGRAM 1. *Cephalotaxus.* In this, and in all the following text-figures, the protoxylem is indicated by the black areas, the metaxylem by dots, and the phloem by diagonal shading.

Transition. At the cotyledonary node the bundles retain their undivided nature and gradually pass inwards towards the central region of the hypocotyl, during which passage the centripetal wood dies out. The arrangement of the vascular bundles at the top of the hypocotyl is, roughly, elliptical; the two seed-leaf-traces occupy the foci of the ellipse, and there are two groups of plumular strands, three in each group, placed at right angles to the cotyledonary plane (Diag. 1, Fig. 1). On tracing the bundles downwards, they undergo a centripetal displacement, so that they soon form a continuous vascular ring by the coalescence of the phloem-masses (Diag. 1, Fig. 2).

The abundant protoxylem of the cotyledonary bundles still occupies an

[1] Worsdell.: On Transfusion Tissue: its Origin and Function in the Leaves of Gymnospermous Plants (Trans. Linn. Soc. London, 2nd ser., vol. v).

endarch position. Very soon the phloem-ring becomes thinner and breaks at the two points opposite the protoxylems of the seed-leaf-traces; but, before this rupture is actually accomplished, each xylem-mass derived from a cotyledon bifurcates, and the protoxylem undergoes a certain amount of rotation, which, however, is not very pronounced and is unequal in degree in different instances; in fact the movement results in nothing much further than the concentration of the protoxylem units in the plane of the cotyledons.

The completion of the division of the vascular tissues results in the exposure of the protoxylems, and in the formation of two well-defined masses of phloem. Concurrently, the metaxylems of the seed-leaf-traces fuse with the adjacent wood of the plumular strands; thus the essential features of a root-structure obtain (Diag. 1, Fig. 3). It is to be noted that, at this region, the protoxylem elements are still arranged rather tangentially, but they gradually become aggregated more closely together, forming with the metaxylem a well-defined diarch plate, and separated from the endodermis by a starch-containing parenchymatous tissue about seven cells in thickness (Diag. 1, Figs. 4 and 5).

C. Fortunei and C. drupacea. The available material of these two species was too scanty to trace the sequence of the transition changes in any detail; sufficient, however, was made out to warrant the assumption that they differ in no important feature from the foregoing species.

The older roots show a well-marked *assise de soutien*, a character which has already been described in this, and also in *Taxus* and *Torreya* by Van Tieghem.[1]

Taxus baccata, Linn. The seedling in its external features resembles *Cephalotaxus* and *Podocarpus* (Figs. 3, 3*a*, 3*b*, Plate XXXV); and, further, the structure and transition-phenomena resemble those of *Cephalotaxus*, and have already been described by Strasburger,[2] so that it is only necessary to draw attention to a few minor characters.

The single bundle of each cotyledon exhibits a mesarch structure, but the number of centripetal elements are very few indeed (Fig. 6, Plate XXXV). The rearrangement of the vascular tissues begins at the level of the cotyledonary node by the xylem taking on a V-like arrangement with the protoxylem occupying the apex which is directed outwards; this is quickly followed by the division of the phloem into two parts. During the inward journey these changes become more marked, and, as the metaxylem travels inwards more quickly than the protoxylem, the latter for a time is mesarch in position (Diag. 3, Fig. 4). A continuous vascular ring is not formed as in the case of *Cephalotaxus*. The separate phloem-masses of each coty-

[1] Van Tieghem: Recherches sur la symétrie de structure des plantes vasculaires (Ann. Sci. Nat., Bot., xiii, 1870–1).

[2] Strasburger: Die Coniferen und die Gnetaceen (Jena, 1872).

ledon-trace immediately effect a junction with the corresponding tissue of the plumular strands, and concurrently the metaxylem of the same traces move towards, and fuse with, the wood of the epicotyledonary bundles. Thus the protoxylems of the seed-leaves are left exposed; there is no definite rotation of this tissue; its exarch position is attained chiefly by the movements of the metaxylem; but, at a lower level, there may be made out a rather indefinite rearrangement of the protoxylem elements coupled with a slight centrifugal movement. The resulting root-structure is exactly the same as in *Cephalotaxus*.

Taxus cuspidata, Sieb. & Zucc. So far as our inadequate material indicates, closely resembles *T. baccata*.

Torreya. It is unfortunately impossible to offer any original observations on this genus, since none of the many seeds planted germinated. This, however, is not of much consequence, as the seedling-anatomy has been fully described by Miss Chick,[1] from whose paper the following remarks are culled:—

The seedling is much like *Ginkgo* in its appearance, with two thick and fleshy hypogeal cotyledons which are closely adpressed and may fuse together by their morphologically upper surfaces. The seed-leaves show much variation in size and in form; one may be about half the length of the other, and, as regards shape, they may be sickle-like, tubular, or with a well-marked spathulate apex. Another feature of interest is found in the fact that the cotyledons show a marked tendency to lobing, which has been described also for Cycads and *Ginkgo* by Strasburger.[2] Mrs. Tansley (*née* Miss Chick) looks upon this lobing as being probably due to the space relations within the seeds caused by the ruminated endosperm, and she cites other cases of seeds with a similar endosperm, viz. Palms and *Myristica*, which also have lobed cotyledons; but at the same time it is pointed out that the cotyledons of *Anona* show no tendency to lobing, although the endosperm is ruminated. We shall offer an alternative explanation later on.

As regards the anatomy of the cotyledons, the main features are as follows: Each has a single vascular bundle, which exhibits centripetal wood and transfusion elements. When the seed-leaf is lobed a branch of the bundle enters the lobe, and in some instances the vascular strand showed a tendency to branch.

The transition is, on the whole, fairly rapid. 'The root protoxylem dies out below the cotyledonary node; and the protoxylem which accompanies the cotyledon-trace outwards would seem to have a double origin, one portion . . . which is directly inserted upon the root metaxylem, and the other seeming to belong more intimately to the cotyledon-trace. . . . A possible explanation of the "dying-out" of the root protoxylem is that

[1] E. Chick: The Seedling of *Torreya Myristica* (New Phytologist, vol. ii, 1903).
[2] Strasburger, loc. cit.

the xylem connexion between the root and the cotyledons was made at a time when, and in a region where, elongation had ceased, and possibly growth in thickness was taking place. This would account for a region devoid of spiral and annular elements.'

The root is diarch, with many fibres in the secondary phloem; also an *assise de soutien* obtains. It is thus seen that, as regards the number and structure of the cotyledons and the anatomy of the root, there is a marked similarity to *Cephalotaxus*; and, further, a study of the figures illustrating the above account leads to the opinion that the transition-phenomena of *Torreya* are also comparable to those of *Cephalotaxus*, although masked by the occurrence of secondary thickening.

PODOCARPEAE.

Podocarpus chinensis. The young seedling is illustrated in Figs. 4 and 4*a*, Plate XXXV. The chief feature to be noted is that the two cotyledons are intra-seminal, and carry the seed up above the level of the ground; the resemblance to the corresponding stages in *Cephalotaxus* and *Taxus* is close, but, as only one seed out of a large number germinated, we have no observations to make regarding the phases later than the one figured.

The general structure of the cotyledons, except with regard to the vascular strands, is like that of *Taxus*. Each seed-leaf of *Podocarpus* has two bundles endarch and collateral throughout the whole length of the leaf, and, relatively, widely separated one from the other. Histologically, the vascular bundles are quite normal; there are no fibres as in *Taxus* and *Cephalotaxus*, and the cambuin is fairly active, judging from the crushed appearance of the outer phloem elements (Fig. 7, Plate XXXV). No centripetal wood has been observed; there are, however, a number of transfusion tracheides generally forming lateral expansions from the metaxylem.

Transition. At the level of the cotyledonary node the pair of bundles of each seed-leaf become orientated in such a manner that their xylem-masses become directed obliquely towards one another (Diag. 2, Fig. 1). This orientation becomes more marked during the inward passage. As the central cylinder is reached, the bundles of each pair approach and become connected, one with the other, by a strand of cambiform cells, which are, in all probability, immature phloem elements (Diag. 2, Fig. 2), on the inner side of which a few tracheae may be seen. There is, however, no direct connexion between the corresponding xylem-masses of each pair of seed-leaf-traces. This bridge of phloem speedily disappears, and at the same time the protoxylem elements of each pair of cotyledon-bundles commence to rotate towards each other and outwards, so as to occupy the exarch position. The traces derived from the seed-

leaves now occupy a position at right angles to the plane of the cotyledons, and the metaxylem and phloem of each strand fuse with the adjacent corresponding vascular tissues derived from the plumule (Diag. 2, Fig. 3).

The completion of these rearrangements results in the formation of a diarch root, the further changes, at lower levels, merely consisting of an increase in the number of protoxylem elements and the constitution of a diarch plate (Diag. 2, Fig. 4).

The main feature of difference between this plant and the other

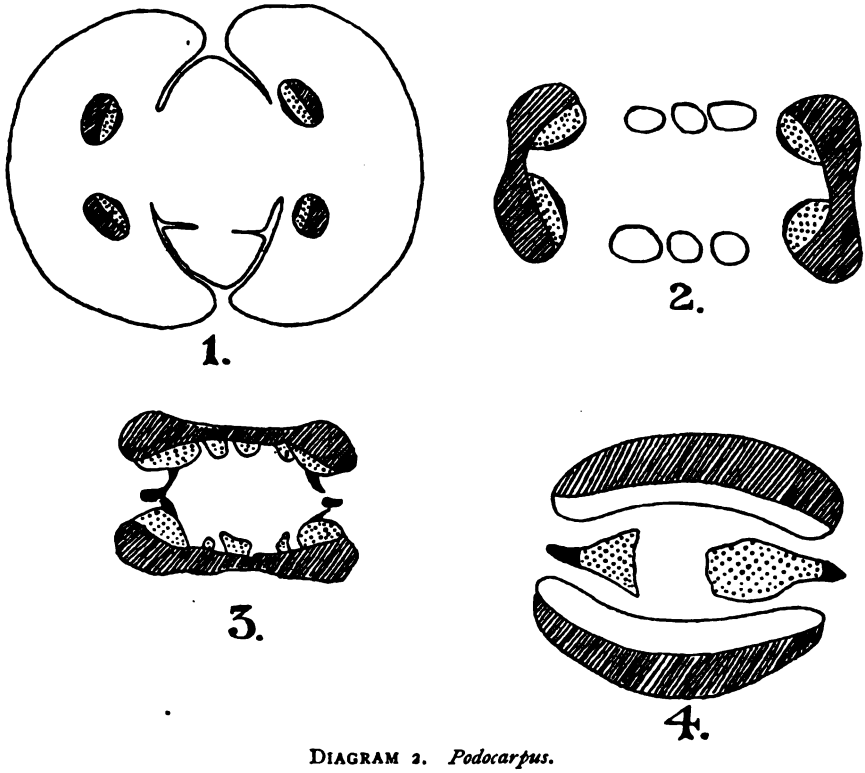


DIAGRAM 2. *Podocarpus.*

members of Taxaceae, so far as they have been examined, is found in the cotyledons, each having two bundles, which together form one pole of the diarch root. As will be seen later on, this feature is characteristic of certain Gnetales.

Owing to failure in obtaining material, it is impossible to say whether the features described above also obtain in the seedlings of other genera of the Podocarpeae. There is, however, a strong indication that such may be the case, for Geyler[1] has pointed out that *Phyllocladus trichomanoides*

[1] Geyler, H. Th.: Einige Bemerkungen über *Phyllocladus*. Abhand. Senckenberg. Naturforsch. Gesellsch., xii, 1881.

has two cotyledons, each of which is traversed by two vascular strands, and Van Tieghem[2] indicates that the primary root of *Phyllocladus* is diarch in structure.

CUPRESSINEAE.

Juniperus virginiana, Linn. The morphology of the seedling in this, and also in the other species examined, calls for no special comment. The epigeal cotyledons are two in number, ligulate in shape, and inserted on the slender hypocotyl, which is continued downwards into the primary root.

Structure of the Cotyledon. Each cotyledon has a single vascular strand, which is collateral throughout its entire length. The bundle is somewhat tangentially elongated, and transfusion tracheides are fairly common, although they are not so abundant in this species as in some others, e. g. *J. Cedrus* (Fig. 8, Plate XXXV). A few xylem elements may occasionally be seen on the ventral side of the protoxylem, hence the cotyledonary bundles are slightly mesarch in structure (cf. Fig. 8, Plate XXXV).

The remaining structural features are identical with those of the seed-leaves of *Taxus* and *Cephalotaxus*, and therefore require no further comment. It may be remarked that no resin canals have been seen in the cotyledons of this and the preceding plants; they are, however, prominent features in the first foliage leaves of *Juniperus*, each having one duct situated immediately below the dorsal ridge (see Diag. 3, Fig. 1).

Transition. The seed-leaf-traces enter the hypocotyledonary axis as collateral structures, and travel obliquely downwards towards the central cylinder (Fig. 9, Plate XXXV). During this passage, which may take place somewhat quickly, the phloem of each bundle bifurcates, leaving the xylem exposed on the dorsal side, and, at the same time, there is a slight rotation of the contiguous half-bundles, which brings the metaxylem elements into a position somewhat more internal than the protoxylem. As a result of this, when the central cylinder is just about reached, each cotyledon-trace has a central mass of xylem, bounded on each side by two separate groups of phloem elements, which lie in a position at right angles to the plane of insertion of the seed-leaves (Diag. 3, Fig. 3).

The protoxylem is almost in an exarch position, but not quite; it is still covered externally by a few metaxylem elements. In other words, the protoxylem, for a time, is in the mesarch position (Diag. 3, Figs. 4 and 5, and Figs. 9 and 10, Plate XXXV). The protoxylem finally becomes exarch by its own somewhat indefinite efforts, aided by the inward movement of the metaxylem. Concurrently, the opposing masses

[1] Van Tieghem, loc. cit.

of phloem of the cotyledonary bundles fuse together either directly, in the case of younger seedlings with no differentiated epicotyledonary

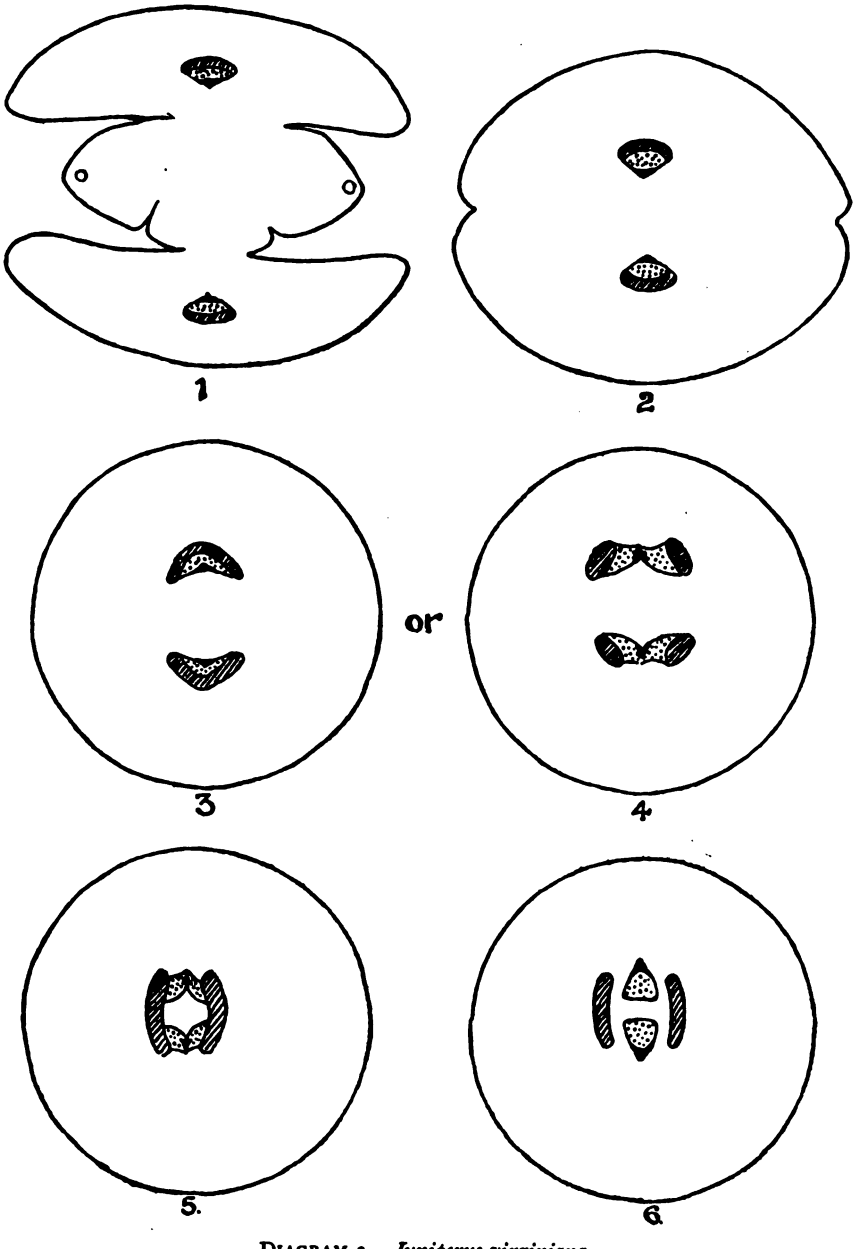


DIAGRAM 3. *Juniperus virginiana.*

traces; or indirectly, by their junction with the intervening plumular bast, as in the case of older seedlings. A diarch root-structure is thus

arrived at in the upper region of the hypocotyl; the endodermis, however, does not appear until the collar is reached. It is to be observed that the protoxylem, during these changes, is always in close contact with the metaxylem; the wood does not divide into two parts, as does the phloem.

A certain amount of variation occurs both in the cotyledonary bundles of one individual and in the transition as seen in different plants of the same species. In the first case, although the bundles behave in a similar fashion, they may not be in precisely the same phase of transition at the same level. In the second case, the relative level in the hypocotyl at which transition begins is not constant, for sometimes the traces reach the central region of the axis and keep to their collateral condition for some little time (Diag. 3, Fig. 2, and Fig. 9, Plate XXXV). The actual mode of rearrangement of the vascular tissues is, however, the same as has been described above. Further, an occasional xylem element may be differentiated well outside the cotyledonary protoxylem after the latter has attained, or nearly attained, its exarch position.

J. Cedrus, Webb & Benth. The structure and transition-phenomena of the seedling of this plant are practically identical with those of *J. virginiana*. The main features of difference, which are of relatively small importance, are as follows: the vascular bundles of the cotyledons of *J. Cedrus* are relatively larger, the tangential expansion is somewhat greater, the transfusion-tissue is more abundant, and the mesarch position of the protoxylem is more marked than in *J. virginiana* (Fig. 8, Plate XXXV). The number of centripetal elements, however, is not great.

Another feature of difference between these two species is that the transition usually begins at a lower level in *J. Cedrus*; but, as regards this point, the same variations obtain as have been described for *J. virginana*.

J. procera in all respects resembles *J. virginiana*; attention, however, may be drawn to the occurrence in one individual of a well-marked bifurcation of the phloem and rotation of the xylem of one of the cotyledon-traces immediately on entering the axis; no other plant of this order, so far as has been seen, exhibited these changes at so high a level as in this particular individual. The other cotyledonary strand behaved in the manner illustrated in Diag. 3, Fig. 3.

J. bermudiana, Linn. The material of this species available for the purposes of this investigation was inadequate, and also unsuitable owing to the presence of much secondary thickening. Sufficient, however, was made out to indicate that this species does not differ in any essential feature from the foregoing. The size of the cotyledon-traces and the paucity of centripetal wood corresponds with what obtains in *J. virginiana*, while the abundance of transfusion elements is in close agreement with *J. Cedrus*. It is, however, not desired to lay any particular stress on this last point, for it is a feature which may vary with the age of the leaf.

Before passing on, attention may be drawn to another feature of resemblance to *Cephalotaxus* and *Taxus* which is found in the presence of a well-marked *assise de soutien* in the roots of *Juniperus*. This is especially well marked in *J. bermudiana*, where the thickenings extend from the layer of cortical cells immediately bounding the endodermis, outwards towards the periphery.

The thickenings of the walls of the outer cells are much smaller than those of the elements nearer the endodermis.

Cupressus obtusa, C. Koch. All the seedlings examined had two cotyledons, which, as regards their form and structure, closely resemble *Juniperus*. The only features of difference are that, in the plant under consideration, transfusion elements are less numerous and the vascular strand has but one or two centripetal tracheae; the bundle is, therefore, but very slightly mesarch.

Transition. The changes which lead to root-structure need no description, inasmuch as they are similar to those exhibited by *Juniperus virginianum*. It is only necessary to remark that the attainment of the exarch position by the protoxylem of the cotyledon-traces is rather more obscure than in any species of *Juniperus* examined; indeed, it is practically impossible to trace the movement of the protoxylem elements, owing to the compact nature of the xylem-masses. The apparent outward passage of these tracheae is considerably aided by the disappearance of some of the metaxylem, and by the inward movement of the rest of these elements. The transition is fairly rapid when once the central cylinder has been reached, so that a root-structure obtains in the higher regions of the hypocotyledonary axis.

Cupressus Lawsoniana, A. Murray. The number of cotyledons and their structure is the same as in *C. obtusa*. The only feature of difference is that in *C. Lawsoniana* the bundles are somewhat larger and, towards the base of the seed-leaves, are more tangentially expanded.

Transition. The transition-phenomena more closely resemble those of *Juniperus virginiana* than do those of *C. obtusa*, for the rearrangement of the wood, culminating in the exarch position of the protoxylem, is more definite. This redistribution usually takes place after the central region of the axis has been reached, but in one instance it was found that the bundle of one of the cotyledons underwent bifurcation of the phloem and partial rotation of the xylem during the inward passage from the seed-leaf. There is thus a similar variation in this plant to that already described for species of *Juniperus*.

Cupressus pisifera, C. Koch., in all respects resembles *C. Lawsoniana*.

Cupressus torulosa, D. Don. The number of cotyledons varies from three to five; thus out of twelve plants, nine had three, two had four,

and one had five seed-leaves. The structure of the cotyledons does not differ from that of the preceding species.

Transition. Considering first an example with three seed-leaves, it

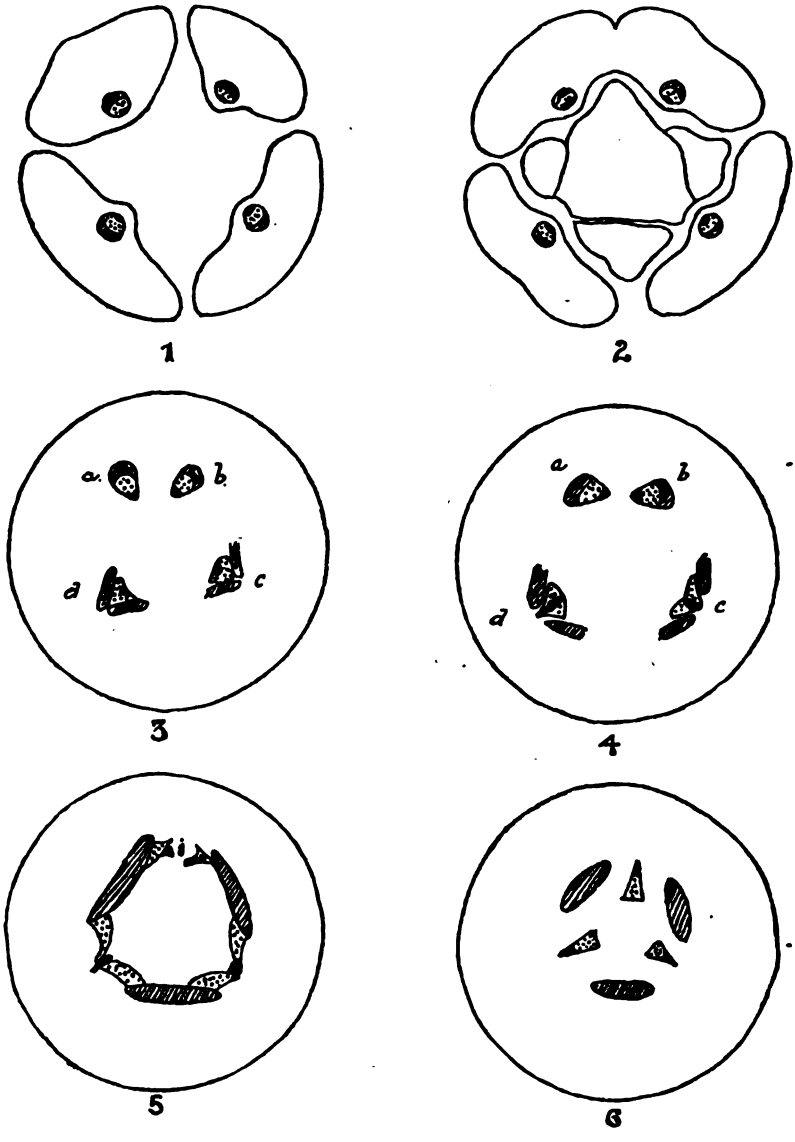


DIAGRAM 4. *Cupressus torulosa.* For the sake of clearness the vascular bundles of the plumule seen in Fig. 2 have been omitted in the later figures.

is found that there is a marked resemblance to the *Juniperus*-type; and, further, there are the same variations as regards the phases of the different bundles at the same level in one and in different individuals.

In the case of a seedling with four cotyledons (Series C) the accompanying illustrations (Diag. 4) render a long description unnecessary. The first figure shows four cotyledons in transverse section; of which two are considerably smaller than the others.

Just above the region of insertion of the seed-leaves upon the axis the two smaller cotyledons fuse together (Diag. 4, Fig. 2). The four traces enter the axis as collateral structures, and during the inward passage *a* and *b*, the bundles of the smaller cotyledons, move towards one another while the phloems of *c* and *d* bifurcate (Diag. 4, Fig. 3). At a slightly lower level (Diag. 4, Fig. 4) *a* and *b* are orientated in such a manner that their protoxylems face one another, and *c* and *d* are seen to behave in a way already described above. The remaining figures, together with Fig. 11, Plate XXXV, show that the strands *a* and *b* act together in the

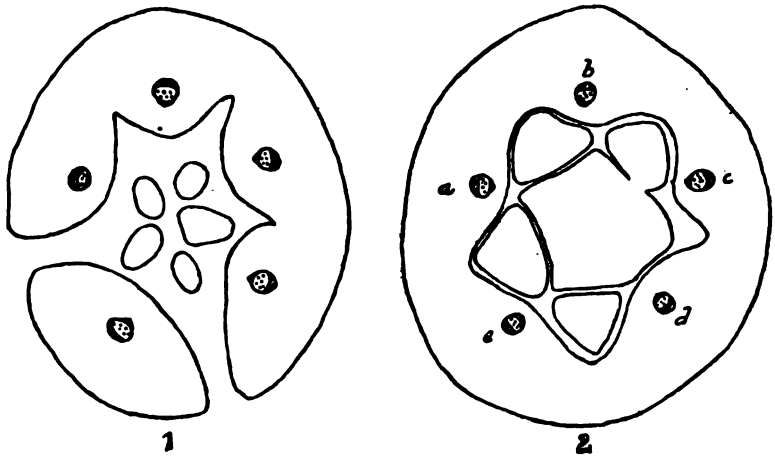


DIAGRAM 5. *Cupressus torulosa.* Transverse sections of the cotyledons at different levels of the proximal region.

same way as does either *c* or *d* separately, and form one pole of the triarch root.

The consideration of these facts leads to the inference that the two smaller seed-leaves may have been derived from a single pre-existing one; in fact, that each represents a half-cotyledon. And this is confirmed by the one seedling of this same species which possessed five cotyledons (Series D). In this plant the seed-leaves were free at the tip, but towards the proximal end four of them joined together in the manner indicated by the first figure in Diag. 5. Just above the cotyledonary node, the fifth one entered and completed the ring. The fused whorl has five bundles, corresponding to the seed-leaves, which are indicated alphabetically in the second figure.

The transition, although very much slower, followed the course

already indicated; the bundle marked *a* formed one pole of the root, *b* and *c* formed the second, while the two remaining bundles, *d* and *e*, formed the third.

The strands *b* and *c*, and *d* and *e*, acted in the same fashion as the bundles *a* and *b* in the seedling with four cotyledons (Diag. 4). It may therefore be concluded, from the behaviour of the vascular bundles, that of the five seed-leaves one (*a*) represents a whole cotyledon, while the rest are half-cotyledons, although a second complication has been introduced by the fusion of several of the seed-leaves.

Cupressus macrocarpa, Hartweg. The number of seed-leaves is three or four; their structure is the same as in the foregoing species, but they are very much narrower, their shape in transverse section being similar to that of the half-cotyledons of *C. torulosa* (Diag. 4, Fig. 1).

Transition. The seed-leaf traces, in a plant with three cotyledons (Series A), behave like bundles *c* and *d* of *C. torulosa* (Diag. 4); that is to say, the phloem of each strand bifurcates during the inward passage and the protoxylem begins to take up the exarch position. These movements are soon completed at a lower level in the hypocotyl, and thus a triarch root results.

In one seedling examined, having four cotyledons (Series B), the transition took place on exactly the same lines as that described for that plant of *C. torulosa* (Series C) which also had four seed-leaves (Diag. 4), the only difference being that in *C. macrocarpa* each of the four cotyledons was quite freely inserted on the axis. Thus of the four seed-leaves, two are equivalent to half-cotyledons, the remaining two being whole cotyledons. It may here be stated that the evidence for the splitting of the seed-leaves is much more abundant in the normally polycotyledonous plants of the Abietineae, and will be dealt with fully later on.

Libocedrus decurrens, Torr. Two seedlings only were available for the purposes of this investigation, and of these one alone was suitable for the study of the transition-phenomena, owing to the presence of extensive secondary thickening in the other. The older of these had two cotyledons, while the younger, which is considered below, had three (Fig. 5, Plate XXXV); Professor Lawson informs us, however, that the usual number of seed-leaves is two.

Although the structure and transition is of the same type as has been described above, the transition from stem to root-structure is very much slower than in any of the preceding plants. The upper part of the hypocotyledonary axis exhibits stem-structure, and the rearrangement of the vascular tissues is very gradual. The phloem of any one bundle diverges on each side of the wood and fuses with the corresponding masses of bast derived from the adjacent bundles; hence a radial arrangement of the essential tissues results. The redistribution of the elements of the

xylem groups, to bring the protoxylem into an exarch position, is very slow. The protoxylem lies buried in the centre of the metaxylem for some distance downwards, and the metaxylem elements slowly take up a more internal position; but this does not bring the protoxylem, which is gradually becoming less in amount, into the desired position, owing to the differentiation of new xylem elements outside it, which addition is much more marked than that described for *Juniperus virginianum*. Indeed, a careful study of these tracheae downwards towards the root apex, warrants the conclusion that in *L. decurrens* the protoxylems of the cotyledons die out and that new protoxylems are organized in an exarch position, which means that the protoxylems of the root are not directly continuous with those of the seed-leaves.

A similar state of affairs has been described in *Torreya Myristica*[1], but at present it is not possible to state whether this occurrence is normal for *Libocedrus*; it is not unlikely that, bearing in mind the variations mentioned, the plant here described may be somewhat abnormal.

Thuja sphaeroidea, Spreng. All the seedlings of this and of the other species examined had two cotyledons. The transition and other features resemble *Juniperus virginianum* in all respects.

Thuja orientalis, Linn., is almost indistinguishable from *Th. sphaeroidea*, the only difference noted being that in *Th. orientalis* the bifurcation of the phloem of the seed-leaf bundles begins directly the inward passage of the strands commence.

Thuja orientalis var. *aurea* and *Th. japonica* are essentially similar to *Th. sphaeroidea*.

Actinostrobus pyramidalis, Miq. Owing to our failure in obtaining material, our observations on this plant are based upon some preparations made by the late Mr. Robertson Glasgow, and kindly placed at our disposal by Dr. D. H. Scott.

Actinostrobus pyramidalis resembles *Thuja orientalis*; the difference between them may be looked upon as one of degree rather than of kind. The bundles of the cotyledons, which are two in number, are single collateral structures throughout the whole length of each seed-leaf, but directly the axis is entered each trace divides into two half-bundles, the division being in the cotyledonary plane. This bifurcation extends not merely through the phloem but also affects the metaxylem. Rotation takes place around the protoxylem, so that the latter tissue is practically in an exarch position as soon as the central region of the hypocotyl is reached (Diag. 6, Fig. 1), the metaxylem and phloem-masses being placed on either side. Fusion of the opposing groups of vascular elements, other than protoxylem, takes place, thus resulting in the formation of a diarch root (Diag. 6, Fig. 2).

[1] Chick, E., loc. cit.

Callitris. All the species of this genus examined had two cotyledons and a primary diarch root.

Callitris Muelleri, Benth. & Hook. resembles *Thuja orientalis.* The transition-phenomena are identical, and, as regards the structure of the cotyledons, there is the same indication of a few centripetal xylem elements in the vascular strands of some individuals; these elements, both in this and in other species, are very poorly marked indeed. Transfusion tissue in all the species examined is fairly abundant.

Callitris calcarata, R. Br., is like the foregoing species, but the phloem of each cotyledon-bundle bifurcates towards the base of each seed-leaf. The protoxylem assumes the exarch position in the same manner as obtains in *Juniperus*, i.e. the metaxylem passes inwards more rapidly than does the protoxylem, and there is a disappearance of some of the

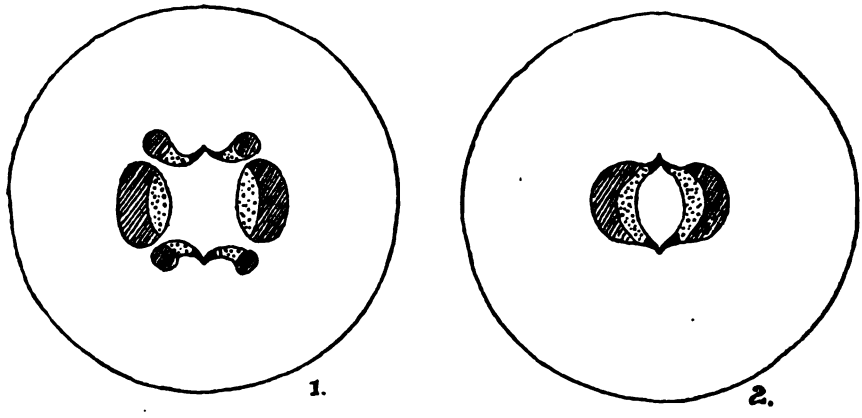


DIAGRAM 6. *Actinostrobus pyramidalis.*

more externally situated metaxylem elements. The assumption of the exarch position of the protoxylem, both in this and in the above species, takes a relatively long time.

Callitris robusta, R. Br., closely resembles *C. calcarata.* The vascular strand of a cotyledon of one individual showed not only a bifurcation of the phloem, but also a division of the wood, accompanied by a certain amount of rotation of the protoxylem to take up the exarch position, while the bundle was still contained within the seed-leaf. The trace of the other cotyledon behaved in the manner described for *C. calcarata.*

In all the species of *Callitris* examined there are the same variations regarding the difference in the phase of each seed-leaf bundle at the same level in the same and in different individuals that have already been mentioned in the case of *Juniperus* and *Cupressus.*

A minor point connected with the foliage leaves may here be remarked upon.

It has been seen that in *Cupressus torulosa* and *C. macrocarpa* there

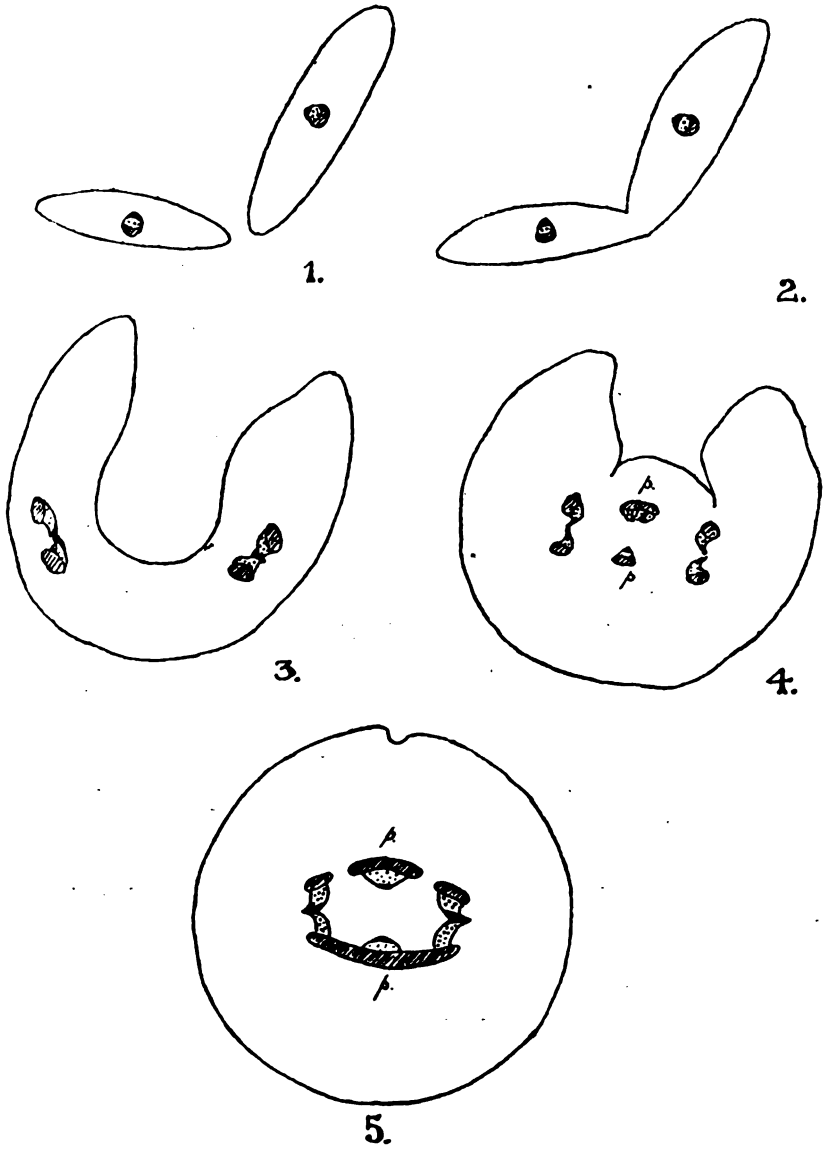


DIAGRAM 7. *Widdringtonia Whytei.* A specimen showing fusion of the cotyledons. The bundles marked *p* are plumular traces.

is evidence which leads to the conclusion that some of the seed-leaves really represent half-cotyledons; one plant of *Callitris robusta* showed

a similar state of affairs in the first whorl of foliage leaves. In this, and in the other plants dealt with, the general rule is that the number of the leaves in the first foliar whorl is equal to the number of cotyledons, and that the number of those in the second foliar whorl of the plumule is double the number of seed-leaves. This particular seedling of *C. robusta* had the usual number of leaves at the first plumular node, but one of them was very distinctly and unequally bifid at the apex; further, the leaf, instead of having the normal single vascular bundle, had two, perfectly distinct throughout, so that a transverse section had the appearance usually presented by a similar section of the double leaves of *Sciadopitys*.

It may, of course, be argued that this is an example of the fusion of two leaves, but on the whole we are of the opinion that it is an instance of the bifurcation of a single leaf.

Callitris rhomboidea, R. Br., resembles *Actinostrobus* as regards the splitting of the metaxylem and rotation of the protoxylem of the cotyledon-traces, during the journey towards the centre of the hypocotyl. The rotation of the protoxylem is, however, not quite of so definite a character as in *Actinostrobus*.

Widdringtonia Mahoni, Mast., shows a slight advance to what obtains in *C. robusta*. A bifurcation of the phloem and metaxylem in each of the bundles of the two cotyledons takes place within the seed-leaves, and is accompanied by a rotation of the protoxylem, which varies in amount in different individuals, and also in the cotyledons of the same plant. This rotation is completed during the inward passage of the seed-leaf-traces to the centre of the hypocotyl, so that a diarch root quickly results.

Widdringtonia Whytei, Rendle, is practically identical, as regards the features under consideration, with *W. Mahoni*. The rotation of the protoxylem is, if anything, more strongly marked in *W. Whytei*, and takes place at a somewhat higher level in each of the cotyledons.

One plant of this species deserves more special mention, inasmuch as it exhibited a fusion of the two cotyledons, strongly recalling the appearance of the foliage leaves of *Sciadopitys*. The seed-leaves were quite free at the tips, but at a slightly lower level they fused together by their edges in the manner indicated in the accompanying diagram (7). The transition took place in the manner already described, and illustrated in Diag. 7.

ABIETINEAE.

TAXODIINAE.

Sequoia sempervirens, Endl. The number of cotyledons, as far as has been seen, is two; and structurally they do not differ in any marked degree from those of the foregoing plants, excepting in the fact that, in

this plant, each seed-leaf has three resin ducts situated just below the epidermis, one at each end of the leaf, and the other immediately above and dorsal to the vascular bundle.

The changes leading to the root-structure are initiated during the inward passage of the traces from the cotyledons, and resemble those obtaining in *Juniperus virginianum.*

Fig. 12, Pl. XXXV, represents a rather curious feature in the seed-leaf bundles in one plant of this species. Each strand entered the axis without any signs of bifurcation of the phloem or rearrangement of the xylem. A cambium extended first on one side of each bundle and then on the opposite side, the activity of this meristem, in each cotyledon-trace, gave rise to an almost concentric vascular strand.

A more remarkable abnormality occurred in another individual. After the transition had been practically effected, meristems arose towards the inner sides of each xylem mass (Diag. 8, Fig. 1). These cambiums effected a junction so that a semi-circular strand occurred on the inner side

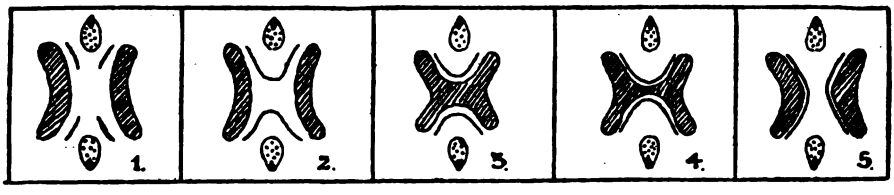


DIAGRAM 8. *Sequoia sempervirens.* Black lines represent cambium, the other markings, as before.

of each bundle of wood-elements (Diag. 8, Fig. 2). The opposing groups of phloem then underwent a gradual centripetal displacement, met in the centre of the axis, and gave rise to an arrangement indicated in the third figure of Diag. 8. The cambium formed a few phloem elements and parenchyma cells, but not in a quantity sufficient to cause crushing (Fig. 13, Plate XXXV). On tracing the tissues still further downwards, the phloem bridge thinned out, and finally broke; thus the normal disposition of tissues was reattained (Diag. 8, Figs. 4 and 5).

Sequoia gigantea, Lindl. & Gord. The number of seed-leaves is usually three or four; structurally they resemble *S. sempervirens,* excepting that only one resin duct is present in each, that on the dorsal side of the vascular strand; also, the transfusion tissue is somewhat more abundant than in *S. sempervirens.*

Taking first a tricotyledonous specimen, it was found that the transition was identical with what obtains in *Widdringtonia Whytei,* the main feature being that bifurcation of the phloem and rotation of the xylem to assume the exarch position, takes place while the bundles

are still contained within the seed-leaves. A triarch root-structure therefore is formed at a very high level of the hypocotyledonary axis.

A plant with four cotyledons gave rise to a tetrarch root in the same manner, although three of the seed-leaf bundles did not start the rearrangement of their vascular tissues until entry into the axis had been gained, and, further, the rotation of the xylem was not nearly so well marked as in the plant of this species first considered.

Another individual with four cotyledons (Series C), afforded further evidence of the splitting of these structures. For the sake of clearness in description the bundles of these seed-leaves may be termed *a*, *b*, *c*, and *d*. *a* underwent bifurcation of the phloem and partial rotation of its xylem while still within the seed-leaf, and ultimately gave rise to one pole of the triarch root. *b* performed in a similar fashion, but did not start the rearrangement of the vascular tissues until its entry into the axis; it also formed one pole of the root. *c* and *d* followed on the same lines as *b*, but the protoxylem of *c* died out, its metaxylem effected a junction with the metaxylem of *d*, the isolated phloem-masses fused, so that a triarch root resulted. There was thus a transient tetrarch arrangement which became reduced to triarch.

From the behaviour of these bundles it must be concluded that *a* and *b* are derived from two whole cotyledons, and as *d* gives rise to the remaining pole of the triarch root it also may be looked upon as belonging to a whole seed-leaf. The remaining strand *c* plays no important part in the formation of the root-structure, and therefore falls into the category of seed-leaves which we have termed subsidiary cotyledons.[1] It is to be observed, however, that this bundle at first behaved in such a fashion as to lead one to suppose that it would form a pole of the root; for bifurcation of the bast and a certain amount of rearrangement of the wood took place. This occurrence is not uncommon and is more especially found in species of *Pinus* for instance; the explanation of it may perhaps be left until these other examples have come under consideration.

Cryptomeria japonica, D. Don. The number of seed-leaves varies from two or three, each has two resin ducts situated one at each end of the leaf.

The transition takes place in the same manner as in the plants already described, resembling *Juniperus virginianum* pretty closely.

All the tricotyledonous plants examined had triarch primary roots.

Sciadopitys verticillata, Sieb. & Zucc., is constant in its number of seed-leaves, never more than two having been observed; they resemble in structure those of *Sequoia sempervirens*, although there is a larger number of transfusion tracheides present; in fact this tissue is more abundant in this plant than in any of the foregoing.

[1] Hill and de Fraine: British Assoc., Section K, York, 1906.

The transition is the same as in *Sequoia gigantea*; a diarch root is, however, always formed.

It may also be remarked that the same variations in the phase of transition of the cotyledonary bundles at the same level occur in this plant as have already been described above for species of *Juniperus*, *Cupressus*, *Callitris*, and *Sequoia*. This is illustrated by Figs 14 and 15, Plate XXXV.

SUMMARY AND CONCLUSIONS.

CQTYLEDONS.

1. As regards the number of cotyledons, the following plants have two:—*Taxus*, *Cephalotaxus*, *Podocarpus*, *Juniperus*, *Cupressus obtusa*, *C. Lawsoniana*, *C. pisifera*, *Thuja*, *Actinostrobus*, *Callitris*, *Widdringtonia*, *Sequoia sempervirens*, and *Sciadopitys*.

The following have 2–3:—*Libocedrus decurrens* and *Cryptomeria japonica*.

The following have 3–4:—*Cupressus macrocarpa* and *Sequoia gigantea*.

The following has 3–5:—*Cupressus torulosa*.

2. Some of the polycotyledonous seedlings form a short cotyledonary tube by the lateral union of the seed-leaves in the proximal region, e. g. *Cupressus torulosa*. Occasionally the two seed-leaves fuse laterally to form a single member, e. g. *Widdringtonia Whytei*.

3. Resin ducts, as far as has been seen, are absent in the seed-leaves of *Cephalotaxus*, *Taxus*, *Podocarpus*, *Juniperus*, *Cupressus*, *Libocedrus*, *Thuja*, *Actinostrobus*, *Callitris*, and *Widdringtonia*. They are present in the cotyledons of *Cryptomeria*, *Sequoia*, and *Sciadopitys*.

4. With the exception of *Podocarpus* and *Cupressus torulosa*, the seed-leaves each contain a single vascular strand.

Podocarpus has two bundles, entirely separate, in each cotyledon, and the same may occur in *Cupressus torulosa*, which is due, in this particular plant, to the splitting of certain of the seed-leaves.

5. The cotyledon-bundles have a pronounced mesarch structure in *Cephalotaxus*; the phenomenon is much less obvious in *Taxus* and *Juniperus*; and is merely indicated by one or two centripetal xylem elements in *Cupressus obtusa*, *C. macrocarpa*, and *Callitris*.

6. Transfusion tracheides are generally present.

7. Elements of a fibrous nature occur in the bast of *Cephalotaxus*, *Taxus*, and *Thuja*.

TRANSITION-PHENOMENA.

8. The transition of the majority of the Taxaceae and Cupressineae follows Van Tieghem's Type 3. That is to say, the single vascular strand of each seed-leaf undergoes bifurcation of the vascular tissues accompanied

by a rotation of the xylem in order that the protoxylem may be situated in an exarch position. Finally, the opposing phloem-masses unite in pairs; thus a diarch root-structure obtains.

9. There is, however, some variation. There is frequently no definite rotation of the protoxylem, the exarch position being gained rather by the movement of the metaxylem elements, e.g. *Taxus*, *Juniperus virginiana*, *J. Cedrus*, *Cupressus obtusa*, *C. pisifera Libocedrus*, and *Callitris*.

The remaining plants considered exhibit a more definite rotation of the protoxylem.

These variations differ in degree in the several plants, and they merge one into the other. Further, it may happen that the trace of one cotyledon shows practically no movement, of a definite nature, of the protoxylem, while the same tissue of the other seed-leaf may exhibit a well-defined rotation e.g. *J. procera*.

There is also a well-pronounced variation, more especially in the Cupressineae, in the level at which the transition begins, and this not only in different species of the same genus, but in individuals of the same species. The transition-phenomena may start in the cotyledons themselves; or, in the topmost part of the hypocotyl, before the traces have reached the central region; or, finally, it may be further postponed and take place at different levels of the hypocotyl. It is obvious that, in the first two cases, a root-structure obtains practically throughout the whole of the hypocotyledonary axis.

10. The transition-phenomena in some of the polycotyledonous forms described (*Cupressus torulosa*, *C. macrocarpa*, and *Sequoia gigantea*) follow the same general course as is summarized above, but showing certain variations which are due to the increased number of seed-leaves. The consideration of these leads to the inference that some of the cotyledons probably represent the halves of single pre-existing seed-leaves; in other words, that the dicotyledonous condition is the more primitive, and that polycotyledony has been derived from it.

11. *Podocarpus*, and possibly also other plants of the Podocarpeae, differ from the other members of the Taxaceae and the Cupressineae, inasmuch as each of the two cotyledons have two vascular bundles, which together form one pole of the primary diarch root.

Root.

12. An *assise de soutien* occurs in the roots of *Cephalotaxus*, *Taxus*, and *Juniperus*.

13. The following table shows the relations, in the plants examined, between the number and nature of the cotyledons and the root-structure.[1]

[1] Van Tieghem (loc. cit.) states that the primary root of the Taxaceae and Cupressineae is diarch, and is in agreement with the number of cotyledons. Occasionally, when the number of seed-leaves is three, the tap-root is triarch.

Plant.	*Series.*	*Cotyledons.*			*Root.*
		Total.	*Whole Cots.*	*Half Cots.*	
Cephalotaxus pedunculata	all	2	2		2-arch
,, *Fortunei*					
,, *drupacea*					
Taxus baccata					
,, *cuspidata*					
Podocarpus chinensis					
Juniperus Cedrus	all	2	2		2-arch
J. bermudiana					
J. virginiana					
J. procera					
Cupressus obtusa					
C. Lawsoniana					
C. pisifera					
C. torulosa	A	3	3		3-arch
,,	B	3	3		3-arch
,,	C	4	2	2	3-arch
,,	D	5	1	4	3-arch
C. macrocarpa	A	3	3		3-arch
,,	B	4	2	2	3-arch
Libocedrus decurrens	A	3	3		3-arch
Thuja sphaeroidea	all	2	2		2-arch
Th. orientalis					
,, var. *aurea*					
Th. japonica					
Actinostrobus pyramidalis					
Callitris Muelleri					
C. calcarata					
C. robusta					
C. rhomboidea					
Widdringtonia Mahoni					
W. Whytei					
Sequoia gigantea	A	3	3		3-arch
,, ,,	B	4	4		4-arch
,, ,,	C	4	3	1	4→3-arch
S. sempervirens	all	2	2		2-arch
Cryptomeria japonica	all	3	3		3-arch
Sciadopitys verticillata	all	2	2		2-arch

EXPLANATION OF FIGURES IN PLATE XXXV,

Illustrating Mr. T. G. Hill's and Miss de Fraine's paper on the Seedling Structure of *Gymnosperms*.

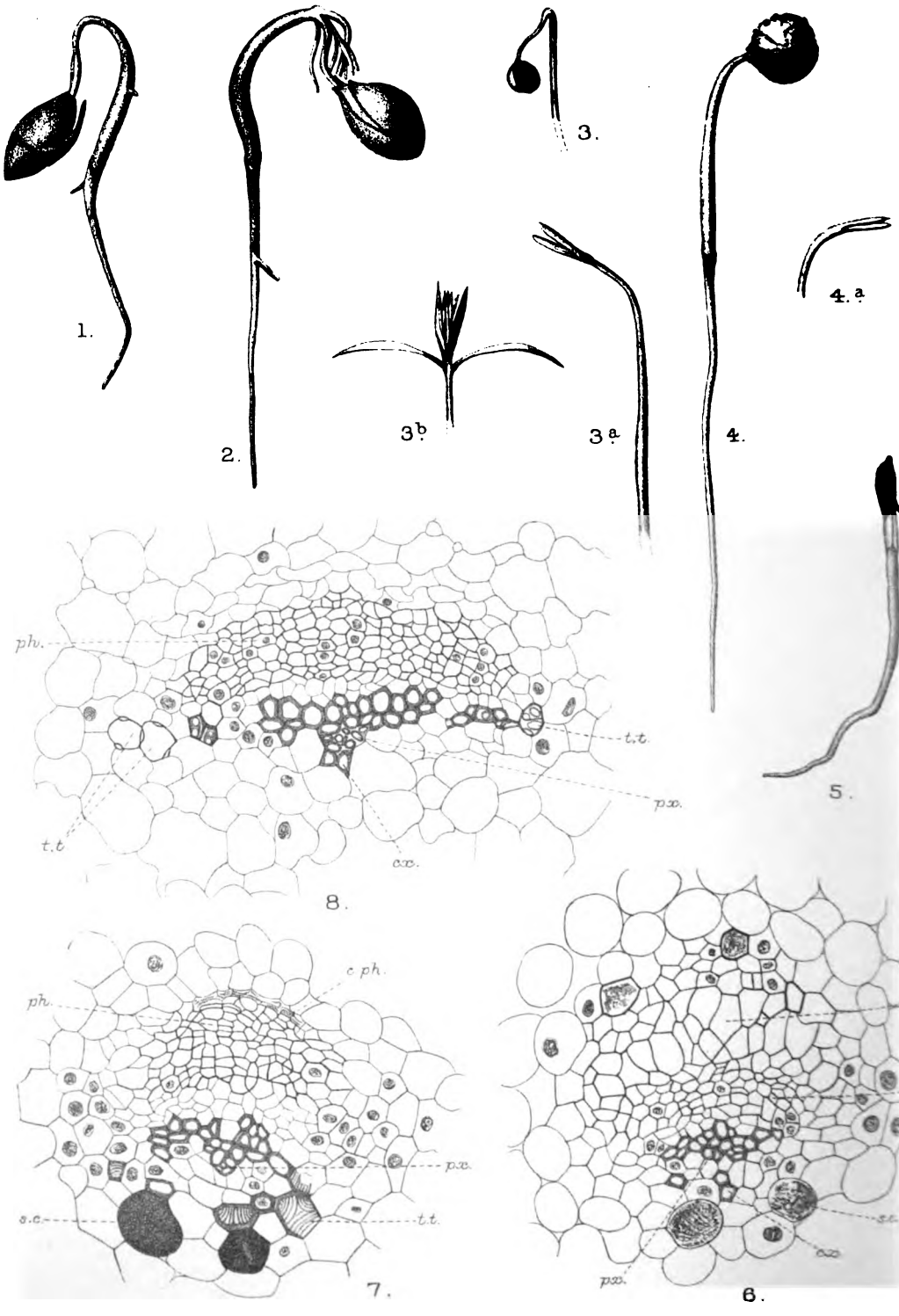
Abbreviations used:—*c.*, cambium; *c. ph.*, crushed phloem; *c. x.*, centripetal xylem; *mx.*, metaxylem; *f.*, fibres; *ph.*, phloem; *px.*, protoxylem; *s. c.*, secretory cells; *T. S.*, transverse section; *t. t.*, transfusion tracheides.

Figs. 1–5 natural size.

Fig. 1. *Cephalotaxus pedunculata.* Young seedling.
Fig. 2. *Cephalotaxus pedunculata.* Older seedling.
Fig. 3. *Taxus baccata.* Upper region of young seedling; cotyledons still enclosed within endosperm.
Fig. 3*a*. *Taxus baccata.* Older seedling; cotyledons free.
Fig. 3*b*. *Taxus baccata.* Still older seedling with plumule.
Fig. 4. *Podocarpus chinensis.* Seedling with cotyledons enclosed within seed.
Fig. 4*a*. *Podocarpus chinensis.* The same seedling showing cotyledons.
Fig. 5. *Libocedrus decurrens.* Young seedling; cotyledons still contained within seed.

Figs. 6–15 × 240.

Fig. 6. *Taxus baccata.* T. S. cotyledon-bundle.
Fig. 7. *Podocarpus chinensis.* T. S. cotyledon-bundle.
Fig. 8. *Juniperus Cedrus.* T. S. cotyledon-bundle.
Fig. 9. *Juniperus virginiana.* T. S. hypocotyl showing seed-leaf-bundle immediately before its bifurcation,
Fig. 10. *Juniperus virginiana.* T. S. hypocotyl, showing the bifurcated cotyledon-bundle nearing the central region of the axis.
Fig. 11. *Cupressus torulosa.* T. S. upper region of hypocotyl.
Fig. 12. *Sequoia sempervirens.* T. S. cotyledon-bundle.
Fig. 13. *Sequoia sempervirens.* T. S. hypocotyl.
Fig. 14. *Sciadopitys verticillata.* T. S. cotyledon-bundle at the extreme base of the seed-leaf.
Fig. 15. The same in another seedling.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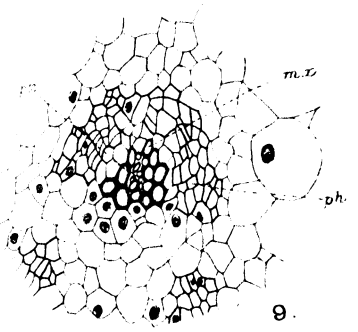

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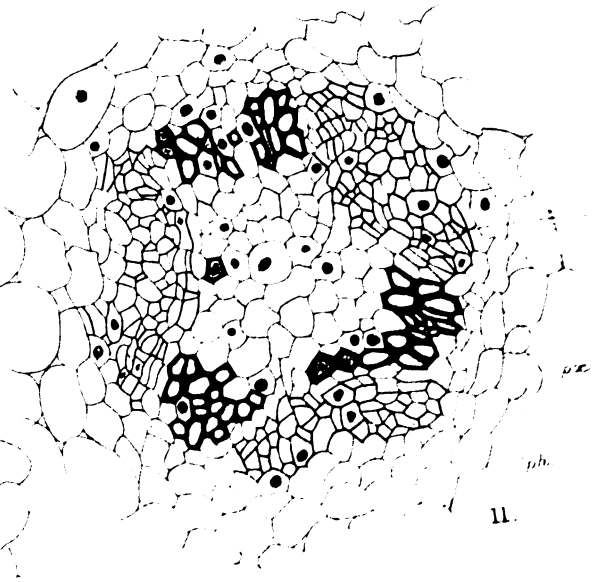

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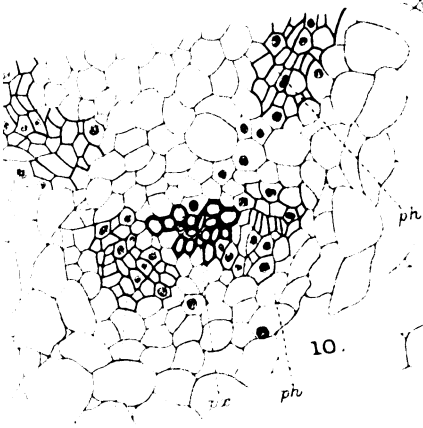

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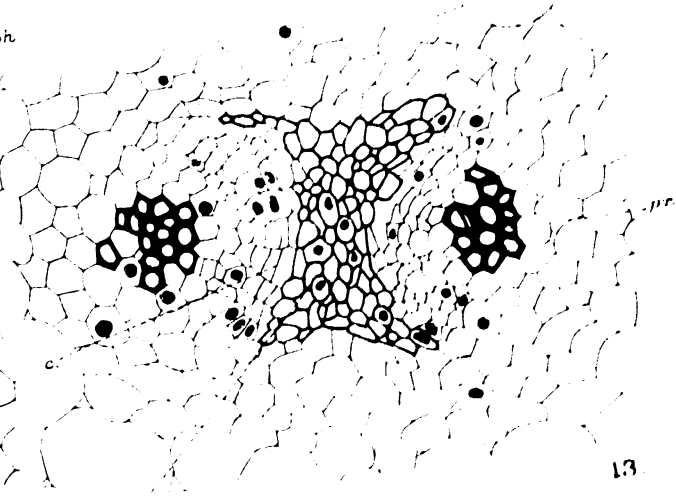

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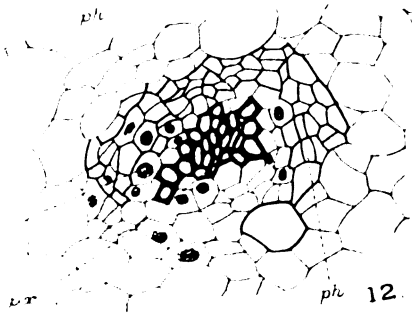

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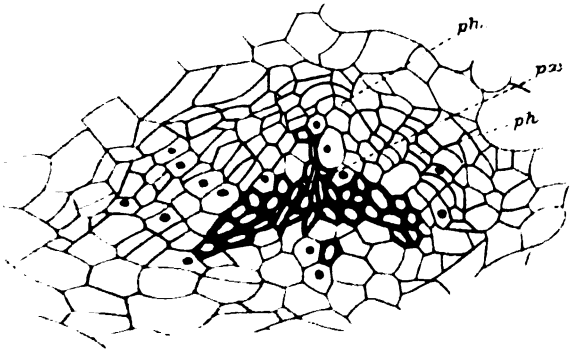

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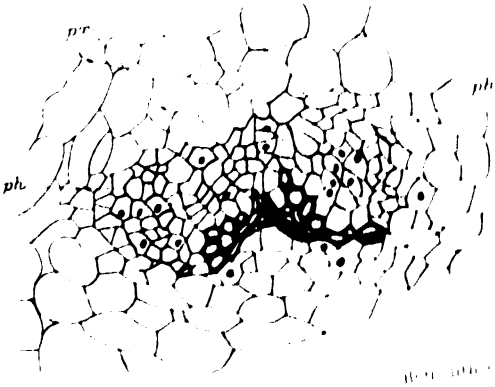

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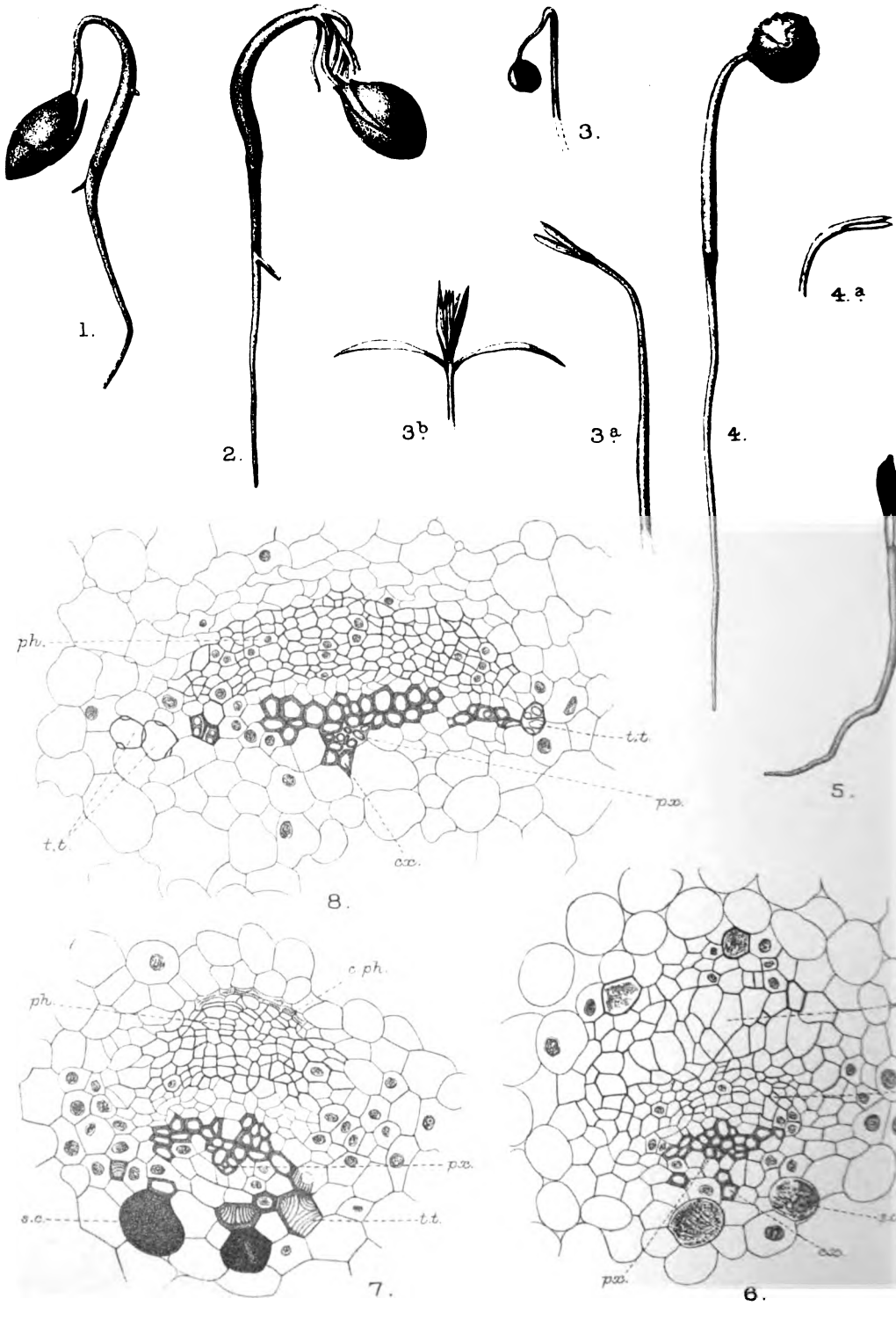
STRUCTURE OF GYMNOSPERMS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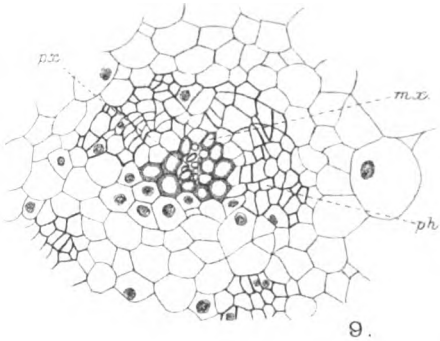

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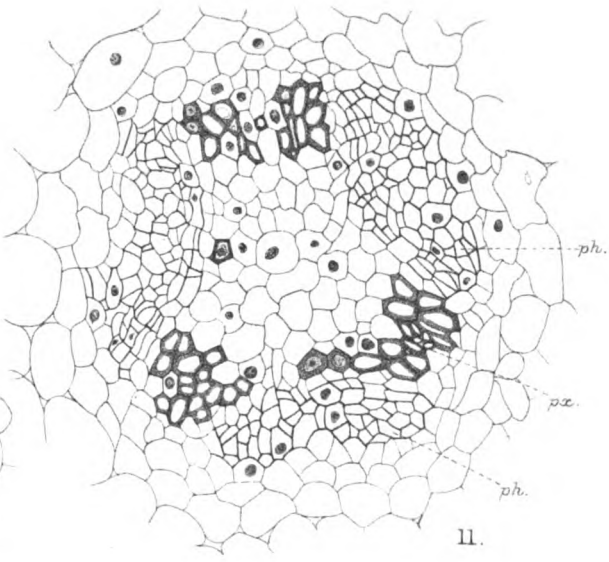

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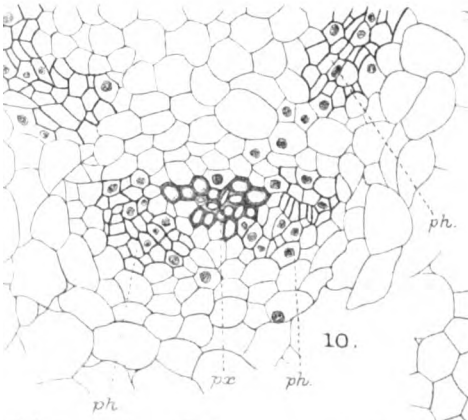

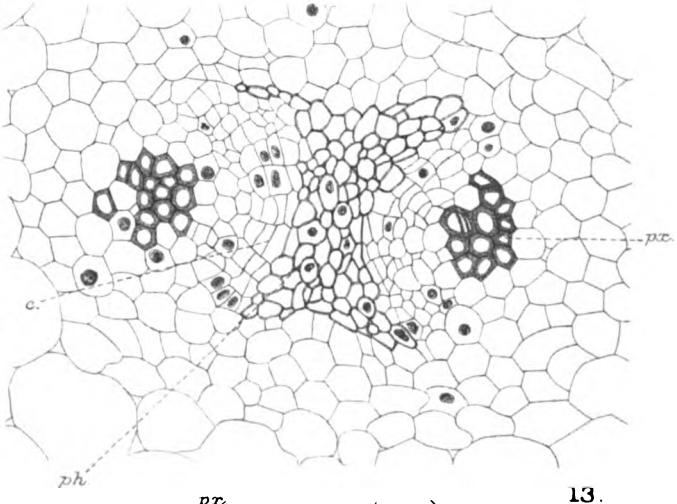

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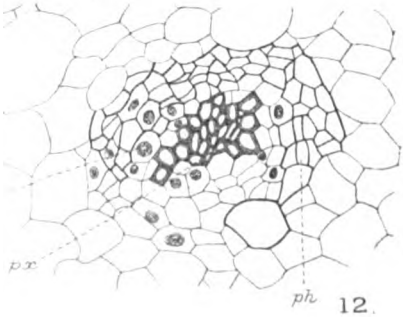

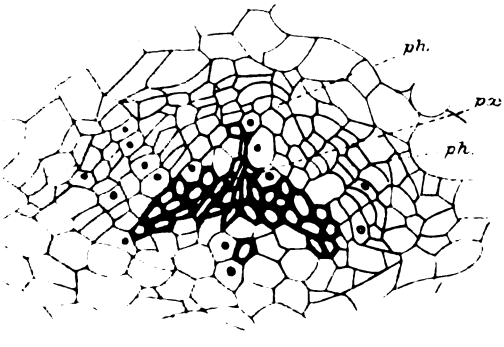

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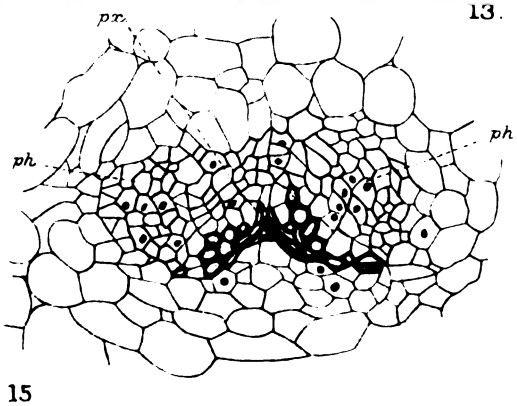

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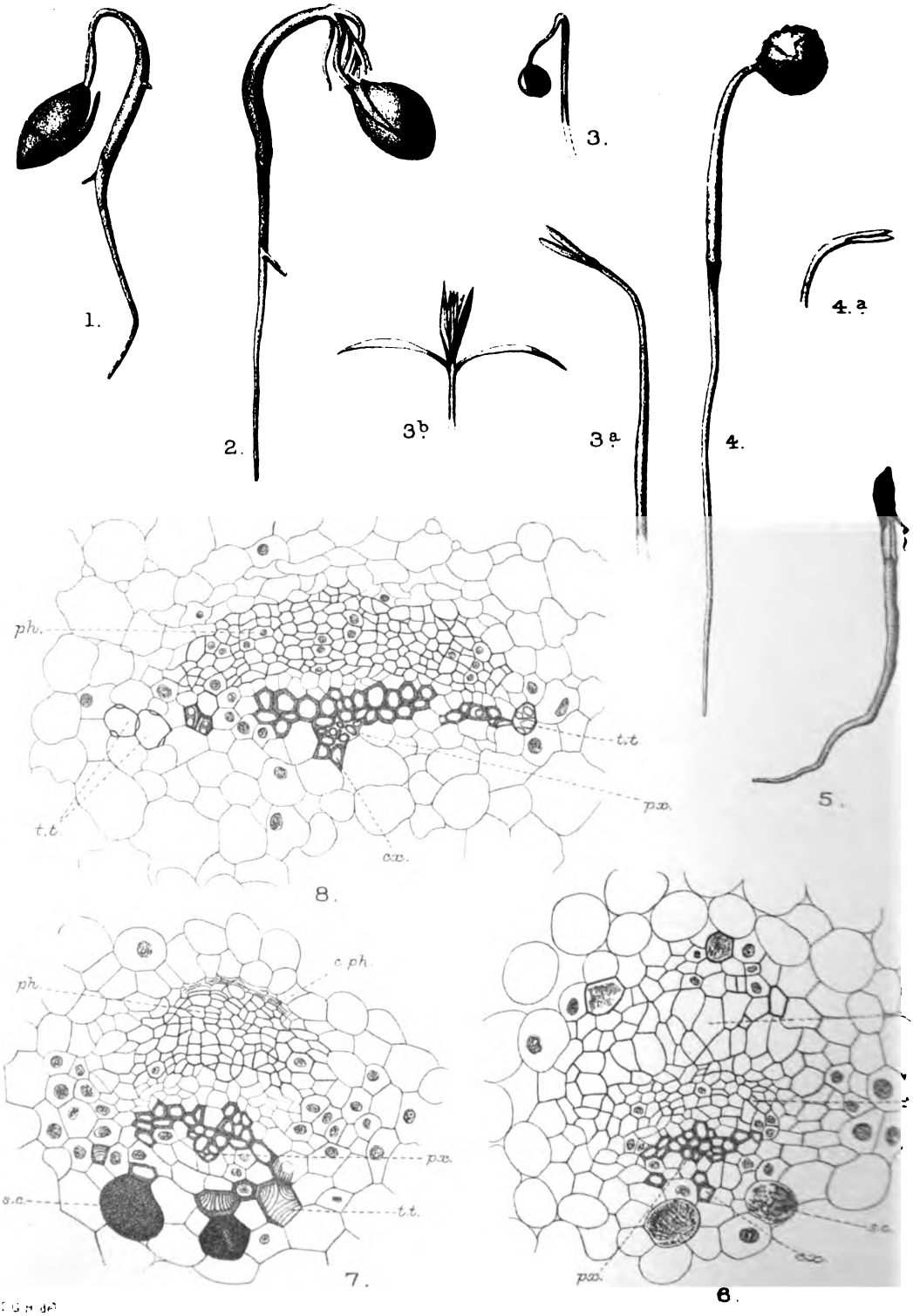
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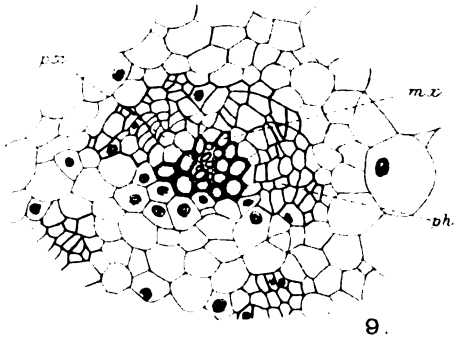

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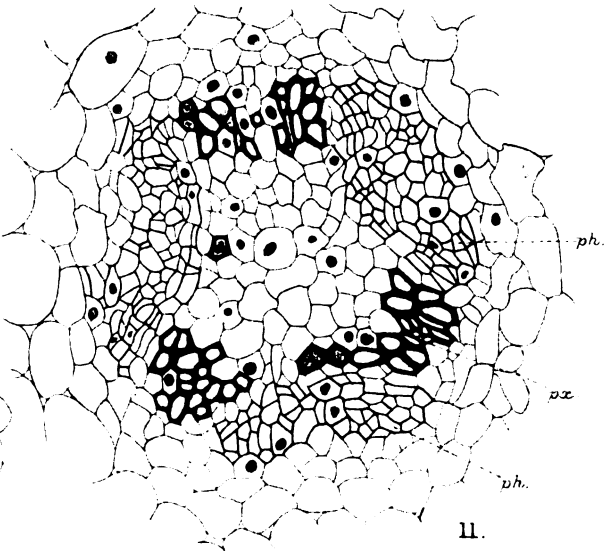

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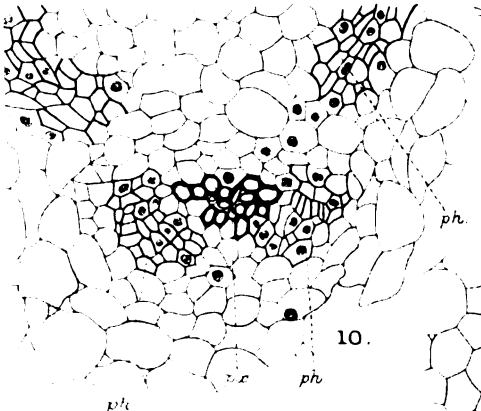

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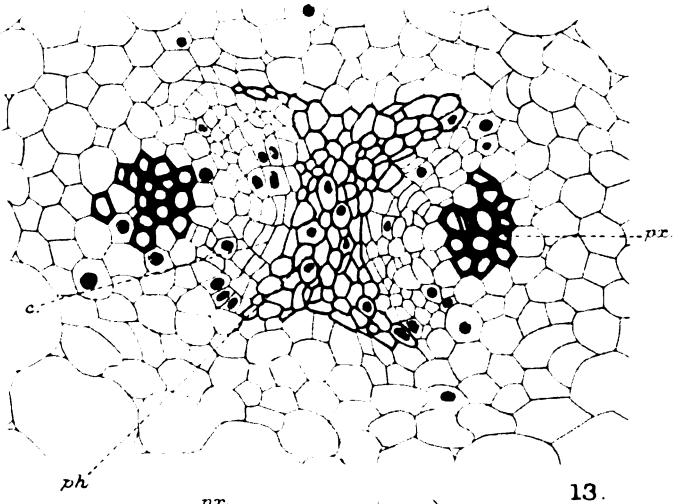

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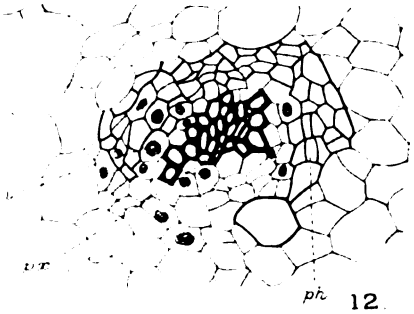

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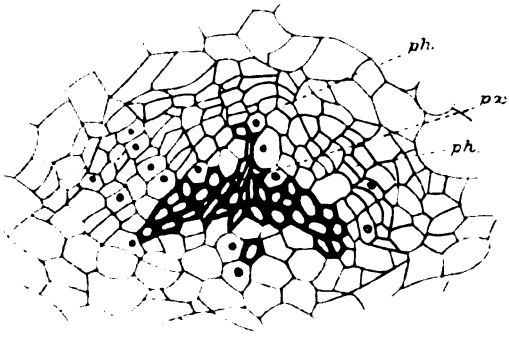

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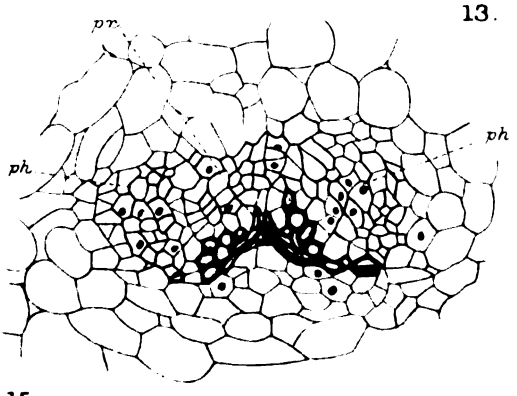

15

FRUCTURE OF GYMNOSPERMS

NOTES.

THE ORIGIN OF MONOCOTYLEDONS.—In the genus *Peperomia*, a perfectly typical and normally dicotyledonous genus, a small group of geophilous individuals has been observed which are peculiar in showing a pseudo-monocotyledonous mode of germination. In this group the embryo is, as I think, without doubt dicotyledonous, but, owing to the assumption of different functions by the two cotyledons, a striking analogy to a monocotyledonous type of embryo has been produced.

In these Peperomias, as a reference to the published figures[1] will show, one of the cotyledons remains within the seed and is entirely a suctorial organ, though it retains, to a certain extent, its dorsiventral structure and possesses rudimentary or rather degenerate stomata. The other cotyledon, however, leaves the seed, a permanent space being left in the seed by its withdrawal, and becomes an assimilating organ and thus assumes the appearance of the characteristic 'first-leaf' of Monocotyledons.

From a careful study of the germination of these geophilous forms, it has been suggested that the possible cotyledonary nature of the 'first-leaf' of Monocotyledons is worthy of some consideration.

In the Bull. Soc. Bot. Fr., sér. iv, vol. viii, 1908, p. 165, MM. Buchet and Gatin describe and figure an interesting case of abnormal germination in *Arisarum vulgare*, Targ.-Tozz. The cotyledon, normally a suctorial organ embedded in the endosperm, appears to have aborted, or perhaps to have been torn off, at an early stage in its development, since traces of torn tissue, which may be interpreted as the remains of the cotyledon sheath, were found at the place where this organ should have occurred. In the absence of the cotyledon the 'first-leaf' had performed the functions of an absorbent organ, for the lamina was found enfolded in the seed and was somewhat thicker than that of the normal 'first-leaf', in consequence, apparently, of its altered functions. The petiole had developed in the usual way, and the seed, with the

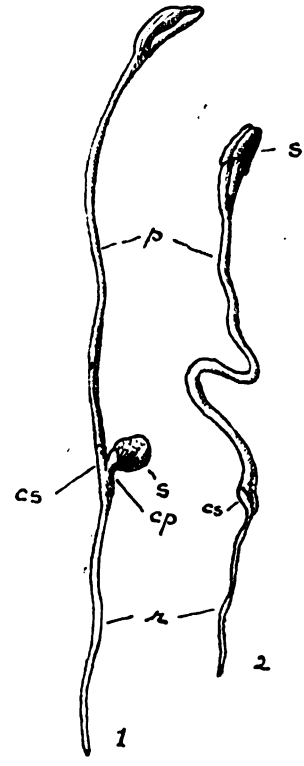

FIG. 1. Normal seedling of *Arisarum vulgare* Targ.-Tozz.

FIG. 2. Abnormal seedling (nat. size): *s.* seed; *p.* petiole of the 'first leaf'; *cp.* petiole of the absorbent cotyledon; *cs.* cotyledonary sheath, represented by a small torn fragment in the abnormal seedling; *r.* radicle. (Copied from Bull. Soc. Bot. Fr., sér. iv, vol. viii, p. 166.)

[1] Hill, A. W., in Ann. Bot., vol. xx, 1906, Pl. XXIX and XXX and vol. xxi, Pl. XV.

included lamina was carried into the air. The 'first-leaf' thus performed the functions of a cotyledon.

This may be considered to be the converse of the state of affairs met with in the geophilous Peperomias.

On the other hand, although without doubt there is an inversion in fact, this case of abnormal germination in *Arisarum* appears to lend support to the argument put forward in connexion with the germination of the Peperomias, since the 'first-leaf' is shown to be physiologically capable of performing cotyledonary functions. Whether or not it is legitimate to argue that in an abnormal case of this kind the 'first-leaf' is really showing a recapitulation of lost functions, and that it is in fact also morphologically the second cotyledon, is an open question.

The morphological relations of the cotyledon and 'first-leaf' in the *Araceae* were pointed out in the paper already referred to,[1] and it does not seem improbable that the 'first-leaf' and the suctorial cotyledon really stand to one another as a pair of cotyledons with different functions. From this point of view the example described by MM. Buchet and Gatin, as far as it may be held to be of value in this connexion, lends support to the views put forward that the 'first-leaf' of Monocotyledons may represent the second cotyledon of the monocotyledonous embryo.

A. W. HILL.

KEW.

ON THE OCCURRENCE OF DIFFERENT TYPES OF HAIR IN THE WALLFLOWER.—The well-known hairs on the leaves of the wallflower are spindle-shaped, with a short stalk at the middle attaching them to the leaf. They may be described as two-armed (malpighian) hairs having the two arms in the same straight line and parallel to the surface of the leaf.[2] Each hair, including its stalk, consists of a single cell, and the surface of the spindle-shaped portion is studded with knobs containing carbonate of lime. Fig. 1, *a* shows the outline of a hair of this type as seen from above, i. e. in a surface-view of the leaf. In a lower focus the stalk would be represented as a small circle in the middle of the hair.

No other type of hair appears to have been recorded in the wallflower, or to occur on the leaf or stem of the mature plant, except on the lower leaves of some lateral branches. On the cotyledons, however, other forms are found, viz. three-armed hairs (Fig. 1, *b*) and hairs with four (Fig. 1, *c*, *d*), five (Fig. 1, *e*) and, rarely, six arms. The two-armed type of hair, characteristic of the mature plant, occurs also on the cotyledons, but is usually far outnumbered by hairs with three or more arms.

On the upper side of the cotyledons the hairs may be many or few, but the proportional number of malpighian hairs (Fig. 1, *a*) is nearly always small. Three-armed hairs appear to be the commonest, but on some cotyledons hairs with four or five arms predominate. On the lower side of the cotyledons the hairs are usually few, and often mostly of the two-armed type, but hairs with three or four arms also occur.

[1] Hill, A. W. in Ann. Bot. xx, pp. 417–422.

[2] Some of the hairs have their arms bent slightly (or, rarely, sharply) upwards, away from the surface of the leaf.

The first two leaves after the cotyledons, when roughly examined with a lens, may appear to bear nothing but malpighian hairs, like the leaves of the mature plant, but more careful examination under a low power of the microscope generally reveals the presence of a small number of three-armed hairs. Some facts connected with the distribution of these hairs are worth recording.

Usually one or more three-armed hairs are to be found on one or both of the first two foliage-leaves, but on the third, fourth, and fifth leaves they are rare, and none were found on later leaves borne by the main axis. A noticeable feature in the distribution of the three-armed hairs is that they are nearly always restricted to the distal half of the leaf, and generally (especially when there are only two or three of them) they lie quite near the apex of the leaf.

Another point of interest lies in the fact that the nature of the soil apparently influences the number and distribution of the three-armed hairs. Twenty seedlings grown in loam were compared with twenty seedlings grown in sand, and the following results were obtained. On the first two leaves, three-armed hairs were decidedly more numerous on the plants grown on sand, the total numbers being 218 for the twenty plants on sand, and 97 for the twenty plants on loam.[1] These figures give an average of about 5·4 three-armed hairs per leaf in the first case, and 2·4 in the second; but the actual numbers vary greatly for different seedlings, thus, while on more that 50 per cent. of these leaves the number of three-armed hairs was 0, 1, or 2, the largest number observed was 34 on sand, and 18 on loam.

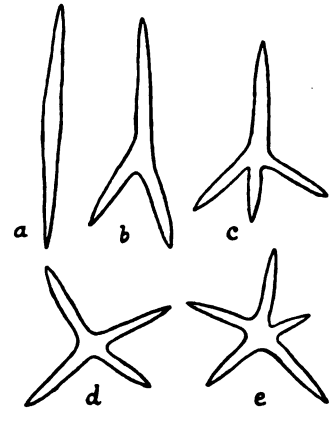

FIG. 1. Some of the types of hair found on the cotyledons of the wallflower. The general outlines are shown without indicating the irregularities caused by the knobs. × 60.

On the two kinds of soil an equally marked difference in the distribution of the three-armed hairs was shown. On loam they were more numerous on the upper side of the leaf (66 upper side, 31 lower), while on sand the numbers on the two sides were nearly equal (110 upper side, 108 lower).

On the third and fourth leaves of the forty seedlings, eleven three-armed hairs were found, three on loam, and eight on sand, while the fifth leaf of twenty plants yielded four of these hairs. Out of the forty seedlings only four (all from loam) showed no three-armed hairs on leaves after the cotyledons.

The conclusion, perhaps, most naturally suggested by the facts described above is that the presence of hairs with three or more arms is an ancestral character preserved in the early stages of the ontogeny of the wallflower, and formerly common to all the foliage-leaves of the plant. This view gains some support from the fact that

[1] On sand the leaves were considerably smaller than on loam, but from some rough estimates it appeared that the total number of hairs (malpighian and three-armed) per leaf was about the same in the two cases, the hairs being more crowded on the smaller leaves.

hairs (with three, four, or more arms) quite similar to those on the cotyledons of the wallflower occur on the leaves of the mature plant in several species of ***Erysimum***, a genus very closely related to *Cheiranthus*.[1]

I hope to publish later some observations on the hairs of the seedlings and mature plants of certain species of *Erysimum*. Further considerations of the results obtained in the wallflower are therefore better postponed.

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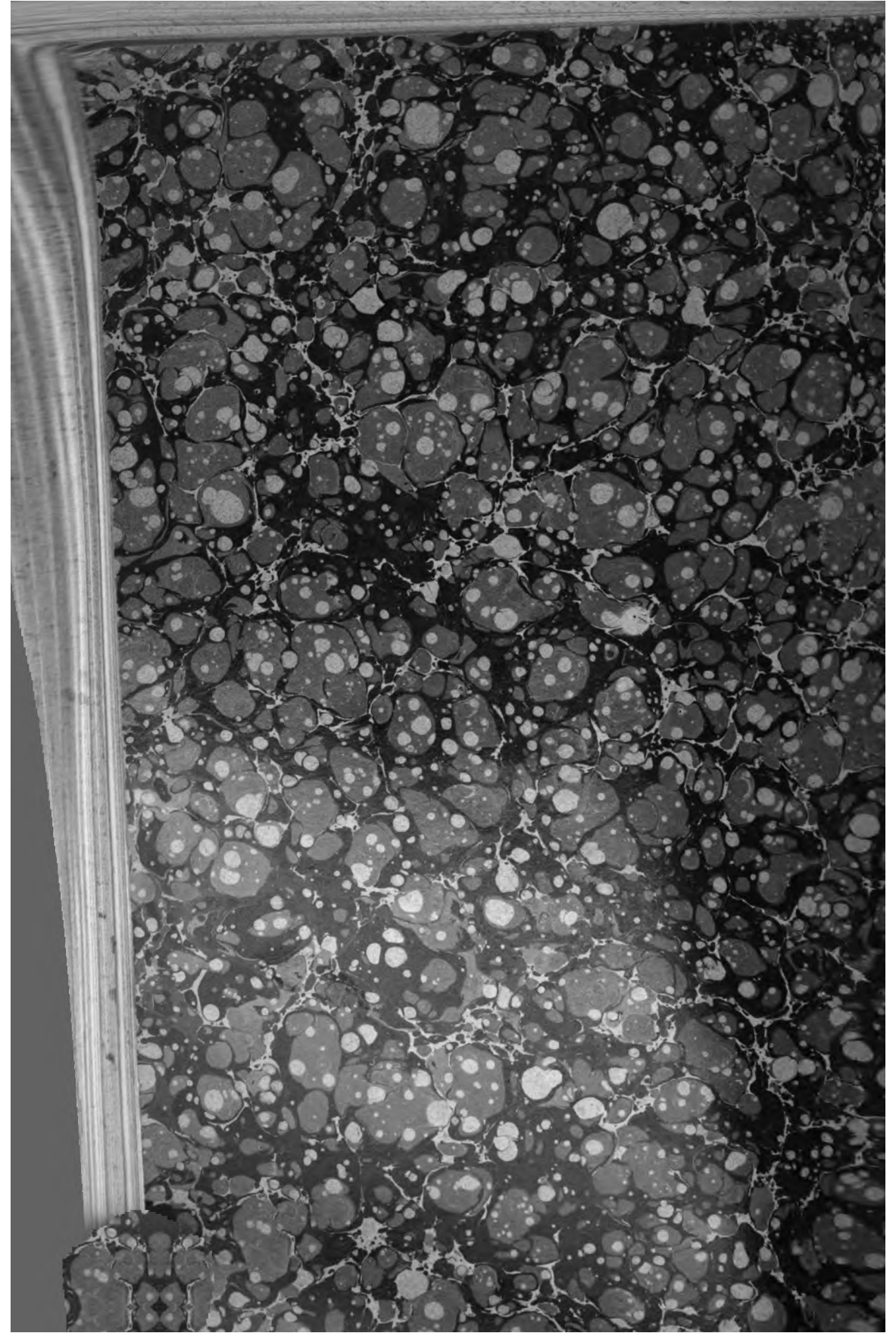
[1] According to Wettstein's observations (Die Gattungen *Erysimum* u. *Cheiranthus*, Oesterreich. Bot. Zeitschrift, 1889, p. 243) the separation of the two genera cannot be upheld.